Centrifuge a portion of the mixture. Use the lower cloudy solution for chromatographic analysis. Filter a few milliliters of the centrifuged solution through an appropriate 2 micron filter.

(C) *Resolution test solution*. Place 15 milligrams each of clindamycin phosphate and clindamycin hydrochloride in a 25-milliliter volumetric flask and dissolve and dilute to volume with mobile phase and mix well. Use this solution to determine the resolution factor.

(iii) *System suitability requirements*—(A) *Asymmetry factor*. Calculate the asymmetry factor ($A_S$), measured at a point 5 percent of the peak height from the baseline as follows:

$$A_s = \frac{a+b}{2a}$$

where:

$a$ = Horizontal distance from point of ascent to point of maximum peak height; and

$b$ = Horizontal distance from point of maximum peak height to point of descent.

The asymmetry factor ($A_s$) is satisfactory if it is not less than 1.0 and not more than 1.3.

(B) *Efficiency of the column*. From the number of theoretical plates ($n$) calculated as described in §436.216(c)(2) of this chapter, calculate the reduced plate height ($h_r$) as follows;

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

$L$ = Length of the column in centimeters;

$n$ = Number of theoretical plates; and

$d_p$ = Average diameter of the particles in the analytical column packing in micrometers.

The absolute efficiency ($h_r$) is satisfactory if it is not more than 15.

(C) *Resolution factor*. The resolution factor ($R$) between the peak for clindamycin phosphate and the peak for clindamycin (hydrochloride) in the chromatogram of the resolution test solution is satisfactory if it is not less than 6.0.

(D) *Coefficient of variation (relative standard deviation)*. The coefficient of variation ($S_R$ in percent) of 5 replicate injections of the working standard solution is satisfactory if it is not more than 2.5 percent. If the system suitability parameters have been met, then proceed as described in §436.216(b) of this chapter.

(iv) *Calculation*. Calculate the clindamycin content as follows:

$$\text{Milligrams of clindamycin per gram} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

$A_u$ = Area of the clindamycin phosphate peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

$A_s$ = Area of the clindamycin phosphate peak in the chromatogram of the clindamycin phosphate working standard;

$P_s$ = Clindamycin activity in the clindamycin phosphate working standard solution in micrograms per milliliter; and

$d$ = Dilution factor of the sample.

(2) *pH*. Proceed as directed in §436.202 of this chapter, using the undiluted cream.

(3) *Identity*. The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the clindamycin phosphate working standard.

[60 FR 49508, Sept. 26, 1995]

# PART 455—CERTAIN OTHER ANTIBIOTIC DRUGS

## Subpart A—Bulk Drugs

Sec.

### Subpart B—Oral Dosage Forms



### Subpart C—Injectable Dosage Forms



### Subpart D—Ophthalmic Dosage Forms



### Subpart E—Otic Dosage Forms



### Subpart F—Dermatologic Dosage Forms



AUTHORITY: Sec. 507 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 357).

SOURCE: 39 FR 19166, May 30, 1974, unless otherwise noted.

## Subpart A—Bulk Drugs

### § 455.4 Aztreonam.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Aztreonam is a practically odorless, white to slightly off-white fine powder. It is sparingly soluble in water of pH 2, and is very soluble at pH values above 4. Its solubility is slight to very slight in polar organic solvents such as methanol and ethanol and it is insoluble in nonpolar solvents such as hexane and heptane. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of aztreonam per milligram on an ''as is'' basis.

(ii) Its moisture content is not more than 2.0 percent.

(iii) Its residue on ignition is not more than 0.1 percent.

(iv) Its heavy metals content is not more than 30 parts per million.

(v) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requirements for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, residue on ignition, heavy metals, and identify.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research; 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 455.4a(b)(1).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(4) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(5) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section, except prepare a solution containing 3 milligrams of aztreonam per milliliter of methanol and use 0.5 milliliter of the solution as the sample.

[54 FR 40385, Oct. 2, 1989]

### § 455.4a Sterile aztreonam.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Aztreonam is a practically odorless, white to slightly off-white fine powder. It is sparingly soluble in water of pH 2, and is very soluble at pH values above 4. Its solubility is slight to very slight in polar organic solvents such as methanol and ethanol and it is insoluble in non-polar solvents such as hexane and heptane. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of aztreonam per milligram on an ''as is'' basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 2.0 percent.

(v) Its residue on ignition is not more than 0.1 percent.

(vi) Its heavy metals content is not more than 30 parts per million.

(vii) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requirements for certification: samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, residue on ignition, heavy metals, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.361 of this chapter, except in lieu of the guard column described in paragraph (a)(4) of that section, use a 5- to 10-centimeter precolumn having an inside diameter of 2 millimeters and packed with octadecyl silane chemically bonded to silica gel of a controlled surface porosity that has been bonded to a solid spherical core (U.S.P. designation L–2) 30 micrograms to 50 micrograms in diameter; and use the resolution test solution to determine resolution in lieu of the working standard solution. Perform the assay at ambient temperature, using an ultraviolet detection system operating at a wavelength of 270 nanometers (or 254 nanometers fixed mercury source), a column packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles (U.S.P. designation L–1) 5 micrograms to 10 micrograms in diameter or equivalent, a flow rate of 1.5 milliliters per minute, and a known injection volume of 20 microliters. Reagents, working standard solution, sample solution, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(*a*) *0.05M potassium phosphate buffer, pH 3.0.* Prepare a solution containing 6.8 grams of potassium phosphate monobasic per liter of distilled water. Adjust the solution to pH 3.0 with 1*M* phosphoric acid.

(*b*) *Mobile phase.* 0.05*M* potassium phosphate buffer, pH 3.0: methanol (4:1).

(ii) *Preparation of working standard, sample, and resolution test solutions*—(*a*) *Working standard solution.* Transfer approximately 25 milligrams of aztreonam working standard, accurately weighed, to a 25-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase.

(*b*) *Sample solution.* Transfer approximately 25 milligrams of the sample, accurately weighed, to a 25-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase.

(*c*) *Resolution test solution.* Dissolve 10 milligrams of [2*S*-[2*alpha*,3*beta*(*E*]]-2-

[[[1-(2-amino-4-thiazolyl)-2-[(2-methyl-4-oxo-1-sulfo-3-azetidinyl)amino]-2-oxoethylidene] amino]oxy]-2-methylpropanoic acid (E isomer) in 10 milliliters of working standard solution and dilute to 50-milliliters with mobile phase.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 2 at 5 percent of peak height.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,000 theoretical plates.

(*c*) *Resolution.* The resolution (*R*) between the peak for aztreonam and the E isomer is satisfactory if it is not less than 2.0.

(*d*) *Coefficient of variation.* The coefficient of variation ($S_R$ in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.361(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(*b*) of this section should not be changed.

(iv) *Calculation.* Calculate the micrograms of aztreonam per milligram as follows:

$$\text{Micrograms of aztreonam per milligram} = \frac{A_u \times P_s}{A_s \times C_u}$$

where:

$A_u$=Area of the aztreonam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

$A_s$=Area of the aztreonam peak in the chromatogram of the aztreonam working standard;

$P_s$=Aztreonam activity in the aztreonam working standard solution in micrograms per milliliter; and

$C_u$=Milligrams of sample per milliliter of sample solution.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid I in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of aztreonam and 39 milligrams of pyrogen-free L-arginine base per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(6) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(7) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section, except prepare a solution containing 3 milligrams of aztreonam per milliliter of methanol and use 0.5 milliliter of the solution as the sample.

[52 FR 4614, Feb. 13, 1987, as amended at 55 FR 11584, Mar. 29, 1990]

### § 455.10 Chloramphenicol.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol is a white to grayish-white or yellowish-white powder, occurring as needles or elongated plates. It is neutral, slightly soluble in water, but freely soluble in alcohol. It has the chemical formula D-(—)-*threo*—1-*p*—nitrophenyl-2 - dichloracetamido-1,3-propanediol. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its pH in a saturated aqueous solution is not less than 4.5 nor more than 7.5.

(iv) Its specific rotation in absolute ethyl alcohol at 20° C. is +20°±1.5°, and at 25° C. is +18.5°±1.5°.

(v) Its melting range is 151°±2° C.

(vi) Its absorptivity at 278 nanometers is 100 ±3 percent of that of the chloramphenicol working standard similarly treated.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay*. Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 95 percent ethyl alcohol to obtain a solution containing 10,000 micrograms of chloramphenicol per milliliter (estimated). Add sufficient distilled water to obtain a concentration of 1,000 micrograms of chloramphenicol per milliliter (estimated). Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *Spectrophotometric method*. Dissolve approximately 50 milligrams each of the sample and working standard in 100 milliliters of distilled water. Warm if necessary to hasten dissolution. Transfer 10 milliliters into a 250-milliliter volumetric flask and fill to volume with distilled water. Using a suitable spectrophotometer equipped with a 1-centimeter cell and distilled water as the blank, determine the absorbance of each solution at 278 nanometers. Calculate the potency of chloramphenicol as follows:

$$\text{Potency of sample in micrograms per milligram} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams}}$$

(2) [Reserved]

(3) *pH*. Proceed as directed in §436.202 of this chapter, using a saturated aqueous solution.

(4) *Specific rotation*. Accurately weigh approximately 1.25 grams of the sample into a 25-milliliter glass-stoppered volumetric flask and dissolve in about 15 milliliters of absolute alcohol, warming if necessary. Dilute the solution to 25 milliliters with absolute alcohol and mix thoroughly. Proceed as directed in §436.210 of this chapter, using a 2.0-decimeter polarimeter tube.

(5) *Melting range*. Proceed as directed in §436.209 of this chapter.

(6) *Absorptivity*. Proceed as directed in paragraph (b)(1)(ii) of this section, except calculate the percent relative absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times 10}$$

(7) *Crystallinity*. Proceed as directed in §436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 45 FR 16476, Mar. 14, 1980; 48 FR 3960, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

### §455.10a Sterile chloramphenicol.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Sterile chloramphenicol is a white to grayish-white or yellowish-white powder, occurring as needles or elongated plates. It is neutral, slightly

soluble in water, but freely soluble in alcohol. It has the chemical formula D - (—) - *threo* - 1 - *p*—nitrophenyl - 2 - dichloracetamido - 1,3 - propanediol. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv)—(v) [Reserved]

(vi) Its pH in a saturated aqueous solution is not less than 4.5 nor more than 7.5.

(vii) Its specific rotation in absolute ethyl alcohol at 20° C. is +20°±1.5°, and at 25° C. is +18.5°±1.5°.

(viii) Its melting range is 151°±2° C.

(ix) Its absorptivity at 278 nanometers is 100 ±3 percent of that of the chloramphenicol working standard similarly treated.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, pH, specific rotation, melting range, absorptivity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 50 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 95 percent ethyl alcohol to obtain a solution containing 10,000 micrograms of chloramphenicol per milliliter (estimated). Add sufficient distilled water to obtain a concentration of 1,000 micrograms of chloramphenicol per milliliter (estimated). Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *Spectrophotometric method.* Dissolve approximately 50 milligrams each of the sample and working standards in 100 milliliters of distilled water. Warm if necessary to hasten dissolution. Transfer 10 milliliters into a 250-milliliter volumetric flask and fill to volume with distilled water. Using a suitable spectrophotometer equipped with a 1-centimeter cell and distilled water as the blank, determine the absorbance of each solution at 278 nanometers. Calculate the potency of chloramphenicol as follows:

$$\text{Potency of sample in micrograms per milligram} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams}}$$

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use 50 milligrams in lieu of 300 milligrams.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams of chloramphenicol per milliliter. Apply sufficient heat to dissolve the chloramphenicol.

(4)—(5) [Reserved]

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated aqueous solution.

(7) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample in a 25-milliliter glass-stoppered volumetric flask and dissolve in about 15

milliliters of absolute alcohol, warming if necessary. Dilute the solution to 25 milliliters with absolute alcohol and mix thoroughly. Proceed as directed in §436.210 of this chapter, using a 2.0 decimeter polarimeter tube.

(8) *Melting range.* Proceed as directed in §436.209 of this chapter.

(9) *Absorptivity.* Proceed as directed in paragraph (b)(1)(ii) of this section except calculate the percent relative absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram}}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times 10}$$

(10) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 45 FR 16476, Mar. 14, 1980; 45 FR 64568, Sept. 30, 1980; 48 FR 3960, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

### §455.11 Chloramphenicol palmitate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol palmitate is the white to grayish-white, tasteless palmitic acid ester of chloramphenicol. It is so urified and dried that:

(i) It contains not less than 555 micrograms nor more than 595 micrograms of chloramphenicol per milligram.

(ii) [Reserved]

(iii) Its melting range is 91°±4° C.

(iv) Its specific rotation in absolute ethyl alcohol at 25° C. is +23°±2°.

(v) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for chloramphenicol content, melting range, specific rotation, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Chloramphenicol content.* Proceed as directed in §436.335 of this chapter.

(2) [Reserved]

(3) *Melting range.* Proceed as directed in §436.209 of this chapter.

(4) *Specific rotation.* Accurately weigh approximately 1.25 grams of sample in a 25-milliliter, glass-stoppered volumetric flask and dissolve in about 15 milliliters of absolute alcohol, warming if necessary to effect solution. Bring the solution to 25° C. Dilute the solution to 25 milliliters with absolute alcohol and mix thoroughly. Proceed as directed in §436.210 of this chapter, using a 2.0-decimeter polarimeter tube.

(5) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 49 FR 6093, Feb. 17, 1984; 50 FR 19921, May 13, 1985]

### §455.12a Sterile chloramphenicol sodium succinate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol sodium succinate is the light-yellow, water-soluble, ethanol-soluble sodium salt of the 3-monosuccinate ester of chloramphenicol. It is so purified and dried that:

(i) Its potency is not less than 650 and not more than 765 micrograms per milligram. If it is packaged for dispensing, its potency when reconstituted as directed in the labeling is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of chloramphenicol per milliliter that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv)—(v) [Reserved]

(vi) Its moisture content is not more than 5.0 percent.

(vii) Its pH in an aqueous solution containing 250 milligrams of chloramphenicol per milliliter is not less than 6.4 and not more than 7.0.

(viii) Its specific rotation in an aqueous solution containing 50 milligrams per milliliter at 25° C. is +6.5°±1.5°.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, and specific rotation.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 500 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 8 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Working standard.* Dissolve an accurately weighed portion of the chloramphenicol working standard in sufficient distilled water to give a solution containing 20 micrograms per milliliter. Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of this solution in a 1-centimeter cell at a wavelength of 278 nanometers.

(ii) *Procedure.* Dissolve an accurately weighed portion of the sample to be tested in sufficient distilled water to give a solution containing 30 micrograms of the sample per milliliter (estimated); and also if it is packaged for dispensing, reconstitute as directed in the labeling. Remove an accurately measured representative portion from each container and further dilute this portion with sufficient distilled water to give a concentration of 20 micrograms of chloramphenicol per milliliter (estimated). Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of this solution in a 1-centimeter cell at a wave length of 276 nanometers. Calculate the micrograms per milligram of the dry powder as follows:

$$\text{Micrograms of chloramphenicol per milligram} = \frac{\text{Absorbance of sample at 276 nanometers} \times \text{micrograms of standard per milliliter} \times \text{potency of chloramphenicol working standard in the micrograms per milligram}}{\text{Absorbance of standard at 278 nanometers} \times \text{micrograms of sample per milliliter}}$$

Calculate the milligrams per milliliter of the reconstituted solution in the dispensing container as follows:

$$\text{Milligrams per milliliter of the reconstituted vial} = \frac{\text{Absorbance of sample at 276 nanometers} \times \text{micrograms of standard per milliliter} \times \text{labeled content of reconstituted vial in milligrams per milliliter}}{\text{Absorbance of standard at 278 nanometers} \times 20}$$

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(a) of this chapter, using a solution containing 5 milligrams of chloramphenicol per milliliter.

(4)—(5) [Reserved]

(6) *Moisture.* Proceed as directed in §436.201 of this chapter.

(7) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 250 milligrams of chloramphenicol per milliliter.

(8) *Specific rotation.* Dilute the sample with sufficient distilled water to give a solution containing approximately 50 milligrams per milliliter. Proceed as directed in §436.210 of this chapter, using a 1.0-decimeter polarimeter tube. Calculate the specific rotation on the anhydrous basis.

[39 FR 19166, May 30, 1974, as amended at 39 FR 37486, Oct. 22, 1974; 45 FR 64568, Sept. 30, 1980; 50 FR 1504, Jan. 11, 1985; 50 FR 19921, May 13, 1985]

### §455.15 Clavulanate potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clavulanate potassium is the potassium salt of *Z*-(2*R*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. It is so purified and dried that:

(i) It is equivalent to not less than 755 micrograms and not more than 920 micrograms of clavulanic acid per milligram on an anhydrous basis.

(ii) Its moisture content is not more than 1.5 percent.

(iii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 5.5 and not more than 8.0.

(iv) It gives a positive identity test.

(v) Its content of the potassium salt of [3*R*,5*S*]-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-3-carboxylic acid (clavam-2-carboxylate) is satisfactory if it is not greater than .01 percent.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, identity, and clavam-2-carboxylate content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 12 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Clavulanic acid content.* Proceed as directed in §436.351 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength between 220 and 230 nanometers, and a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl silane bonded silica. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(*a*) *0.05M Sodium phosphate buffer solution, pH 4.4.* Transfer 7.8 grams of monobasic sodium phosphate to a 1-liter volumetric flask and dissolve in 900 milliliters of distilled water. Adjust the pH to 4.4±0.1 with 18*N* phosphoric acid or 10*N* sodium hydroxide. Dilute to volume with distilled water. Mix well.

(*b*) *Mobile phase.* Mix methanol: 0.05*M* sodium phosphate buffer, pH 4.4 (5:95 v/v) and stir or ultrasonicate for no less than 2 minutes. Degas by passing through a 0.5-micrometer filter with vacuum. The mobile phase may be sparged with helium through a 2-micrometer metal filter for the duration of the analysis. Adjust the ratio of methanol to aqueous buffer as necessary to obtain satisfactory retention of the peaks.

(ii) *Preparation of clavulanic acid working standard and sample solutions.* Accurately weigh and transfer into volumetric flasks sufficient clavulanic acid working standard or clavulanate potassium sample to obtain a final concentration of 250 micrograms per milliliter. To the clavulanic acid working standard, add sufficient amoxicillin trihydrate to provide a final concentration of 500 micrograms per milliliter. (The amoxicillin serves as an internal marker for system suitability testing.) Dissolve in water by shaking or

ultrasonicating until solution becomes clear. Dilute the solutions as required to final volume with water. Use within 8 hours.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.5.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 550 theoretical plates.

(*c*) *Resolution factor.* The resolution factor (*R*) between the clavulanic acid and amoxicillin peaks is satisfactory if it is not less than 3.5.

(*d*) *Coefficient of variation.* The coefficient of variation ($S_R$ in percent) is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.351(b) of this chapter.

(iv) *Calculations.* Calculate the micrograms of clavulanic acid per milligram of sample as follows:

$$\text{Micrograms of clavulanic acid per milligram} = \frac{A_u \times P_s \times W_s \times 100}{A_s \times W_u \times (100 - m)}$$

where:

$A_u$=The clavulanic acid peak response in the chromatogram of the sample (at a retention time equal to that observed for the standard);

$A_s$=The clavulanic acid peak response in the chromatogram of the clavulanic acid working standard);

$P_s$=Potency of the clavulanic acid working standard in micrograms per milligram;

$W_u$=Milligrams of sample;

$W_s$=Milligrams of standard; and

m=Percent moisture content of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(4) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation described in paragraph (b)(2) of that section.

(5) *Clavam-2-carboxylate content.* Proceed as directed in § 436.352 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 210 nanometers, and a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing materials such as octadecyl silane bonded silica. Mobile phase, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Mobile phase. 0.1M Sodium phosphate buffer solution, pH 4.0.* Prepare a 0.1*M* aqueous solution of monobasic sodium phosphate and adjust to pH 4.0 with phosphoric acid.

(ii) *Working standard and sample solutions*—(*a*) *Preparation of working standard solution.* Accurately weigh and transfer into a 50-milliliter volumetric flask approximately 16 milligrams of clavam-2-carboxylate authentic sample. Dilute to volume and transfer 10 milliliters into a 100-milliliter flask. Dilute to volume with water.

(*b*) *Preparation of sample solution.* Accurately weigh 100 milligrams of the sample into a 10-milliliter flask. Dilute to volume with water.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) for the clavulanate standard peak is satisfactory if it is not more than 1.5.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 4,000 theoretical plates.

(*c*) *Resolution factor.* The resolution factor (*R*) between the clavulanic acid and clavam-2-carboxylic acid peaks is satisfactory if it is greater than 1.0.

(*d*) *Coefficient of variation (Relative standard deviation).* The coefficient of variation ($S_R$ in percent) is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in § 436.352(b) of this chapter.

(iv) *Calculations.* Calculate the percent of clavam-2-carboxylate content as follows:

$$\text{Percent clavam-2-carboxylate content} = \frac{\text{Mean sample height (or area)} \times \text{weight of standard} \times P}{\text{Mean peak height (or area) of standard} \times \text{weight of sample} \times 50}$$

where:

*P*=Percent clavam-2-carboxylic acid in the standard.

[49 FR 39674, Oct. 10, 1984, as amended at 55 FR 11584, Mar. 29, 1990]

### § 455.15a Sterile clavulanate potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Clavulanate potassium is the potassium salt of *Z*-(2*R*,5*R*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. It is so purified and dried that:

(i) It is equivalent to not less than 755 micrograms and not more than 920 micrograms of clavulanic acid per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 1.5 percent.

(v) Its pH of an aqueous solution containing 10 milligrams per milliliter is not less than 5.5 and not more than 8.0.

(vi) It gives a positive identity test.

(vii) Its [3*R*,5*S*]-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-3-carboxylic acid (clavam-2-carboxylate) content is satisfactory if it is not greater than .01 percent.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, identity, and clavam-2-carboxylate content.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 12 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Clavulanic acid content.* Proceed as directed in § 455.15(b)(1) of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 10 milligrams per milliliter of clavulanate potassium.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 10 milligrams per milliliter.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation described in paragraph (b)(2) of that section.

(7) *Clavam-2-carboxylate content.* Proceed as directed in § 455.15(b)(5) of this chapter.

[50 FR 33519, Aug. 20, 1985, as amended at 54 FR 11584, Mar. 29, 1990]

### § 455.20 Cycloserine.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cycloserine is a white to slightly yellowish compound. It has the chemical structure D-4-amino-3-isoxazolidone. It is so purified that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 1.0 percent.

(iv) Its pH in a 10 percent aqueous solution is not less than 5.5 and not more than 6.5.

(v) Its residue on ignition is not more than 0.5 percent.

(vi) It gives a positive identity for cycloserine.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, residue on ignition, crystallinity, and identity.

(ii) Samples of the batch: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Using the cycloserine working standard as the standard of comparison, assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Colorimetric assay*—(*a*) *Stockstandard solution.* Dry approximately 100 milligrams of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight and dissolve the dried working standard in sufficient distilled water to give a solution containing 1,000 micrograms per milliliter.

This solution may be used for 1 month if kept under refrigeration.

(*b*) *Standard curve solutions.* Pipette accurately 0.0, 1.0, 5.0, 10.0, 15.0, and 20.0 milliliters of the stock standard solution to each of six 100-milliliter volumetric flasks, dilute to 100 milliliters with 0.1*N* sodium hydroxide and mix thoroughly.

(*c*) Reagents:

(*1*) Acetic acid—1.0*N* solution.

(*2*) Sodium hydroxide—4.0*N* and 0.1*N* solutions.

(*3*) Sodium nitroprusside—4.0 percent solution: Dissolve 4.0 grams in sufficient distilled water to make 100.0 milliliters. Mix well. Store in amber bottle.

(*4*) Oxidized nitroprusside reagent—Mix equal parts of 4 percent sodium nitroprusside solution and 4.0*N* sodium hydroxide, and let stand for 1 hour before using. Prepare daily, and store in an amber bottle.

(*d*) *Procedure.* Transfer approximately 100 milligrams of sample, accurately weighed, to a 100 milliliter volumetric flask. Dissolve in sufficient 0.1*N* sodium hydroxide to measure exactly 100 milliliters. Mix thoroughly and transfer 10 milliliters to a second 100-milliliter volumetric flask, and mix thoroughly. Transfer exactly 1.0 milliliter of each of the standard curve solutions and of the sample solution to respective test tubes. Add exactly 3.0 milliliters of 1.0*N* acetic acid to each of the test tubes. Mix thoroughly. Add exactly 1.0 milliliter of oxidized nitroprusside reagent to each test tube and mix thoroughly. Allow the tubes to stand at room temperature for at least 10 minutes in order that maximum color intensity may develop. Using the solution containing 0.0 milliliter of working standard as a blank, determine the absorbances of the solutions at 625 nanometers in a suitable spectrophotometer. Plot concentration versus absorbance on linear graph paper. The curve may deviate slightly from a straight line. The standard curve solutions equal 0, 10, 50, 100, 150, and 200 micrograms of cycloserine, respectively.

(*e*) Calculations:

Micrograms cycloserine per milligram = (Concentration in micrograms from calibration curve × 1,000)/Weight of original sample in milligrams.

(ii) *Microbiological turbidimetric assay.* Proceed as described in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute the stock solution with sterile distilled water to the reference concentration of 50 micrograms of cycloserine per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution with a concentration 100 milligrams per milliliter.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(6) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(7) *Identity.* Proceed as directed in paragraph (b)(1)(i) of this section.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

### § 455.40 Mupirocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Mupirocin is nonanoic acid, 9-[[3-methyl-1-oxo-4-[tetrahydro-3,4-dihydroxy-5-[[3-(2-hydroxy-1-methylpropyl)oxiranyl]methyl]-2*H*-pyran-2-yl]-2-butenyl]oxy]-,[2*S*-[2α(*E*),3*B*,4*B*,5α[2*R**,3*R**(1*R**,2*R**)]]]-. It is a white to off-white crystalline solid. It is so purified and dried that:

(i) Its potency is not less than 920 micrograms per milligram on an anhydrous basis.

(ii) Its moisture content is not more than 1.0 percent.

(iii) The pH of a saturated aqueous solution of mupirocin is not less than 3.5 and not more than 4.0.

(iv) It is crystalline.

(v) It gives a positive identity test for mupirocin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, crystallinity, and identity.

(ii) Samples, if required by the Center for Drug Evaluation and Research: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 229 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as an octadecylsilane, a flow rate of not more than 2.0 milliliters per minute, and a known injection volume of between 10 and 20 microliters. Use the resolution test solution to determine resolution in lieu of the working standard solution. Reagents, working standard and sample solutions, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *Acetonitrile.* Distilled in glass. Ultraviolet grade.

(B) *Phosphate buffer, pH 6.3.* Prepare a 0.05*M* sodium monobasic phosphate solution and adjust to pH 6.3 with 1.0*N* sodium hydroxide.

(C) *Mobile phase.* To 750 milliliters of 0.05*M*, pH 6.3 phosphate buffer, add 250 milliliters of acetonitrile. Filter through a suitable filter capable of removing particulate matter to 0.5 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph.

(ii) *Preparation of working standard, sample, and resolution, test solutions*—(A) *Working standard solution.* Accurately weigh approximately 11 milligrams of the mupirocin working standard into a 100-milliliter volumetric flask. Dissolve the standard in about 20 milliliters of acetonitrile and dilute to volume with pH 6.3 phosphate buffer. Mix well.

(B) *Sample solution.* Transfer approximately 11 milligrams of sample, accurately weighed, to a 100-milliliter volumetric flask. Dissolve the sample in about 20 milliliters of acetonitrile and dilute to volume with pH 6.3 phospate buffer. Mix well.

(C) *Resolution test solution.* Acidify approximately 10 milliliters of the working standard solution with 6*N* hydrochloric acid to pH 2.0. Allow to stand at room temperature for about 2 hours. Neutralize this solution. Use this solution to determine the resolution requirement for the chromatographic system.

(iii) *System suitability requirements*—(A) *Asymmetry factor.* Calculate the asymmetry factor ($A_s$), measured at a point 5 percent of the peak height from the baseline as follows:

$$A_s = \frac{a+b}{2_a}$$

where:

$a$=Horizontal distance from point of ascent to point of maximum peak height; and

$b$=Horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor ($A_s$) is satisfactory if it is not more than 1.5.

(B) *Efficiency of the column.* From the number of theoretical plates ($n$) calculated as described in § 436.216(c)(2) of this chapter, calculate the reduced plate height ($h_r$) as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

$L$=Length of the column in centimeters;

$n$=Number of theoretical plates; and

$d_P$=Average diameter of the particles in the analytical column packing in micrometers.

The absolute efficiency ($h_r$) is satisfactory if it is not more than 20.0, equivalent to 1,500 theoretical plates for a 30-centimeter column of 10 micrometer particles.

(C) *Resolution factor.* The resolution factor ($R_s$) between the peak for mupirocin and its nearest eluting peak produced from its acid degradation is satisfactory if it is not less than 2.0. The chromatogram of the resolution test solution should show a significantly reduced mupirocin peak immediately preceded by a peak due to mupirocin degradation products. This

degradation peak may appear as a single peak or be partially resolved showing a shoulder or two overlapping peaks.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation ($S_R$ in percent of 5 replicate injections) is satisfactory if it is not more than 2.0 percent.

If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations.* Calculate the micrograms of mupirocin per milligram of sample as follows:

$$\text{Micrograms of mupirocin per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

$A_u$=Area of the mupirocin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

$A_s$=Area of the mupirocin peak in the chromatogram of the mupirocin working standard;

$P_s$=Mupirocin activity in the mupirocin working standard solution in micrograms per milliliter;

$C_u$=Milligrams of mupirocin sample per milliliter of sample solution;

m=Percent moisture content of the sample.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter using a saturated aqueous solution.

(4) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(5) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in § 436.211(b)(2).

[55 FR 2641, Jan. 26, 1990; 55 FR 11110, Mar. 26, 1990; 55 FR 14378, Apr. 17, 1990]

### § 455.50 Calcium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium novobiocin is the calcium salt of a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 840 micrograms per milligram, expressed in terms of novobiocin on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 10 percent.

(iv) Its pH in a saturated aqueous suspension containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(v) Its specific rotation in an acidmethyl alcohol solution at 25° C. is not less than −50° and not more than −58°.

(vi) It demonstrates a positive color identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, specific rotation, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in 5 milliliters of absolute ethyl alcohol and then dilute with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of 1,000 micrograms (estimated) per milliliter. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated aqueous suspension prepared by suspending 25 milligrams of calcium novobiocin per milliliter.

(5) *Specific rotation.* Proceed as directed in § 455.51a(b)(8).

(6) *Identity.* Proceed as directed in § 455.51(b)(7).

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 41 FR 10886, Mar. 15, 1976; 43 FR 9801, Mar. 10, 1978; 50 FR 19921, May 13, 1985]

### § 455.51 Sodium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin is the monosodium salt of a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms of novobiocin per milligram, calculated on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 6.0 percent.

(iv) Its pH in a solution containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(v) Its residue on ignition is not less than 10.5 percent and not more than 12.0 percent, calculated on an anhydrous basis.

(vi) Its specific rotation in an acidmethyl alcohol solution at 25° C. is not less than −50° and not more than −58°.

(vii) It demonstrates a positive color identity test.

(viii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification: samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, residue on ignition, specific rotation, identity and crystallinity.

(ii) Samples required on the batch; 10 packages, each containing approximately 600 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 25 milligrams of sodium novobiocin per milliliter.

(5) *Residue on ignition.* Proceed as directed in § 436.207(b) of this chapter, calculating on the basis of an anhydrous sample weight.

(6) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample in a 25-milliliter glass-stoppered volumetric flask. Prepare an acid-methyl alcohol solution by diluting 1.0 milliliter of concentrated hydrochloric acid to a volume of 100 milliliters with absolute methyl alcohol and mix well. Dissolve the sample in about 15-milliliters of the acid-methyl alcohol solution. Adjust to volume with the acid-methyl alcohol solution and mix well. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube. Calculate the specific rotation on the anhydrous basis.

(7) *Identity.* (i) Using 0.1*M* aqueous sodium borate as a diluent, prepare 10 milliliters of a solution containing the equivalent of 1 milligram (approximate) of novobiocin per milliliter.

(ii) Prepare a saturated aqueous solution of *N*,2,6-trichloroquinoneimine by shaking continuously for 30 minutes in a dark bottle 25 milligrams of *N*,2,6-trichloroquinoneimine in 100 milliliters of distilled water. Let stand 2 hours after shaking. Store in the dark bottle.

(iii) Add 2.0 milliliters of the saturated *N*,2,6-trichloroquinoneimine solution to 4 milliliters of the novobiocin solution. Mix well and heat in a water bath at 37° C. for 10 minutes. The development of a blue color is a positive test for the presence of novobiocin. To 2 milliliters of the blue solution, add 2 milliliters of *N*-butyl alcohol and shake well. A green color should develop in the butyl alcohol layer. To the other 2-milliliter portion of the blue solution, add 2 milliliters of benzene (c.p.), and shake well. A pink color should be developed in the benzene layer.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

### § 455.51a Sterile sodium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

*and purity.* Sodium novobiocin is the crystalline monosodium salt at a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms of novobiocin per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its loss on drying is not more than 6.0 percent.

(vi) Its pH in a solution containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(vii) Its residue on ignition is not less than 10.5 percent and not more than 12.0 percent calculated on an anhydrous basis.

(viii) Its specific rotation in an acidmethyl alcohol solution at 25° C. is not less than −50° and not more than −58°.

(ix) It demonstrates a positive color identity test.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, residue on ignition, specific rotation, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 600 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(a) of this chapter, using a solution containing 10 milligrams of novobiocin per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(6) *pH.* Proceed as directed in §436.202 of this chapter, using a solution containing 25 milligrams of sodium novobiocin per milliliter.

(7) *Residue on ignition.* Proceed as directed in §436.207(b) of this chapter, calculating on the basis of an anhydrous sample weight.

(8) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample in a 25-milliliter glass-stoppered volumetric flask. Prepare an acid-methyl alcohol solution by diluting 1.0 milliliter of concentrated hydrochloric acid to a volume of 100 milliliters with absolute methyl alcohol and mix well. Dissolve the sample in about 15-milliliters of the acid-methyl alcohol solution. Adjust to volume with the acid-methyl alcohol solution and mix well. Proceed as directed in §436.210 of this chapter, using a 2.0-decimeter polarimeter tube. Calculate the specific rotation on an anhydrous basis.

(9) *Identity.* (i) Using 0.1*M* aqueous sodium borate as a diluent, prepare 10 milliliters of a solution containing the equivalent of 1 milligram (approximate) of novobiocin per milliliter.

(ii) Prepare a saturated aqueous solution of *N*,2,6-trichloroquinoneimine by shaking continuously for 30 minutes in a dark bottle 25 milligrams of *N*,2,6-trichloroquinoneimine in 100 milliliters of distilled water. Let stand 2 hours after shaking. Store in the dark bottle.

(iii) Add 2.0 milliliters of the saturated *N*,2,6-trichloroquinoneimine solution to 4 milliliters of the novobiocin solution. Mix well and heat in a water bath at 37° C. for 10 minutes. The development of a blue color is a positive test for the presence of novobiocin. To 2 milliliters of the blue solution, add 2 milliliters of *N*-butyl alcohol and shake well. A green color should develop in

the butyl alcohol layer. To the other 2-milliliter portion of the blue solution, add 2 milliliters of benzene (c.p.), and shake well. A pink color should develop in the benzene layer.

(10) *Crystallinity*. Proceed as directed in §436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

### §455.70 Rifampin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Rifampin is a red-brown powder. It is 3-(4-methylpiperazinyliminomethyl) rifamycin SV. It is very slightly soluble in water, soluble in ethyl acetate and in methyl alcohol, and freely soluble in chloroform. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 2 percent.

(iv) Its pH is not less than 4.0 and not more than 6.0 in a 1 percent aqueous suspension.

(v) When calculated on the anhydrous basis, its absorptivity at 475 nanometers is 100±4 percent of that of the rifampin working standard, similarly treated.

(vi) It passes the identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a stock solution containing 1.0 milligram of rifampin per milliliter (estimated). Further dilute an aliquot of the stock solution with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 5.0 micrograms of rifampin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter, except dry the sample for 4 hours.

(4) *pH*. Proceed as directed in §436.202 of this chapter, using a 1 percent aqueous suspension.

(5) *Absorptivity*. Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 100 milligrams each of the sample and standard in a 100-milliliter volumetric flask containing 50 milliliters of absolute methyl alcohol, and dilute to volume with absolute methyl alcohol. Transfer a 2-milliliter aliquot to a 100-milliliter volumetric flask, and dilute to volume with 1 percent potassium phosphate buffer, pH 6.0, as listed in §436.101(a)(1) of this chapter. Using a suitable spectrophotometer equipped with a 1-centimeter cell, immediately determine the absorption of each solution at 475 nanometers with the blank containing the same proportion of solution 1 and methyl alcohol as the sample and standard solutions. Calculate the absorptivity as follows:

$$\text{Percent relative absorptivity} = \frac{\dfrac{\text{Absorbance of sample} \times \text{milligrams standard} \times (100 - m_1)}{\text{Absorbance of standard} \times \text{milligrams sample} \times (100 - m_2)}}{} \times 100$$

where:

$m_1$=percent moisture in standard;

$m_2$=percent moisture in sample.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(3) of that section, except use a 4 percent solution of the sample in chloroform and 0.1-millimeter matched absorption cells.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

### § 455.80a Sterile spectinomycin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile spectinomycin hydrochloride is the pentahydrated dihydrochloride salt of decahydro-4a, 7, 9-trihydroxy-2-methyl-6,8-bis(methylamino)4H-pyrano[2,3-b][1,4]benzodioxin-4-one. It is so purified and dried that:

(i) Its spectinomycin content is not less than 603 micrograms per milligram. If it is packaged for dispensing, its spectinomycin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of spectinomycin that it is represented to contain.

(ii) Its microbiological activity is not less than 603 micrograms of spectinomycin per milligram.

(iii) It is sterile.

(iv) It is nonpyrogenic.

(v) [Reserved]

(vi) It contains no depressor substances.

(vii) Its moisture content is not less than 16 percent nor more than 20 percent.

(viii) Its pH is an aqueous solution containing 10 milligrams per milliliter is not less than 3.8 nor more than 5.6. If it is packaged for dispensing, when reconstituted as directed in the labeling, its pH is not less than 4.0 nor more than 7.0.

(ix) It passes the identity test.

(x) Its residue on ignition is less than 1 percent.

(xi) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for spectinomycin content, microbiological activity, sterility, pyrogens, depressor substances, moisture, pH, identity, residue on ignition, and crystallinity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: eight packages, each containing approximately 300 milligrams and two containing not less than 3 grams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Spectinomycin content* (*vapor phase chromatography*). Proceed as directed in § 436.307 of this chapter; and also, if the batch is packaged for dispensing prepare the sample for assay as follows: Reconstitute the vial as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container, or if the labeling specifies the amount of spectinomycin content in a given volume of the resultant preparation remove an accurately measured representative portion from the container. Dilute the sample with water to a concentration equivalent to about 20 milligrams per milliliter of spectinomycin. Transfer 1.0 milliliter of the diluted sample to a 25-milliliter glass-stoppered Erlenmeyer flask and dry by lyophilization. Proceed as directed in § 436.307(d)(1)(ii) of this chapter. Calculate the spectinomycin content as follows:

$$\text{Milligrams of spectinomycin per dose} = \frac{R_u \times W_s \times D \times f}{R_s}$$

where:

$$R_u = \frac{\text{Area of spectinomycin sample peak (at a retention time equal to that observed for the spectinomycin standard)}}{\text{Area of internal standard peak}}$$

$$R_s = \frac{\text{Area of the spectinomycin standard peak}}{\text{Area of internal standard peak}}$$

$W_s$=Weight of the spectinomycin working standard in milligrams;
$D$=Dilution of the spectinomycin dose;
$f$=Potency of the spectinomycin working standard in milligrams of spectinomycin per milligram.

(2) *Microbiological activity* (*microbiological turbidimetric assay*). Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 30.0 micrograms of spectinomycin per milliliter (estimated).

(3) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(4) *Pyrogens.* Proceed as directed in §436.32(a) of this chapter, using a solution containing 50 milligrams of spectinomycin base per milliliter.

(5) [Reserved]

(6) *Depressor substances.* Proceed as directed in §436.35 of this chapter.

(7) *Moisture.* Proceed as directed in §436.201 of this chapter.

(8) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter, except, if it is packaged for dispensing, use the suspension obtained after reconstituting the drug as directed in the labeling.

(9) *Identity test.* Proceed as directed in §436.211 of this chapter, using the method described in paragraph (b)(2) of that section.

(10) *Residue on ignition.* Proceed as directed in §436.207 of this chapter, using the method described in paragraph (b) of that section.

(11) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19921, May 13, 1985]

### §455.82a Sterile sulbactam sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile sulbactam sodium is sodium (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4 dioxide. It is so purified and dried that:

(i) Its sulbactam potency is not less than 886 micrograms and not more than 941 micrograms per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) Its moisture content is not more than 1 percent.

(v) It is crystalline.

(vi) It passes the identity test for sulbactam sodium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, crystallinity, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 30 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 230 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silica, a flow rate of 2.0 milliliters per minute, and a known injection volume of 10 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *1.0M Phosphoric acid.* Prepare by dissolving 67.5 milliliters of reagent grade phosphoric acid (85 percent) in distilled water and dilute to 1 liter.

(B) *0.005M Tetrabutylammonim hydroxide.* Dilute 6.6 milliliters of tetrabutylammonium hydroxide (40 percent) to 1,800 milliliters with distilled water. Adjust the pH to 5.0 with 1.0*M* phosphoric acid and dilute with distilled water to 2 liters.

(C) *Mobile phase.* Mix 350 milliliters of acetonitrile with 1,650 milliliters of 0.005*M* tetrabutylammonium hydroxide. Filter and degas the mobile phase just prior to its introduction into the chromatographic pumping system. (Slight adjustments in pH and/or acetonitrile content may be made to achieve the system suitability parameters defined in paragraph (b)(1)(iii) of this section.)

(ii) *Preparation of working standard and sample solutions*—(A) *Working standard solution.* Dissolve an accurately weighed portion of sulbactam working standard in sufficient mobile phase to give a stock solution of a known concentration containing about 1 milligram of sulbactam per milliliter.

(B) *Sample solution.* Dissolve an accurately weighed portion of the sample in sufficient mobile phase to give a stock solution containing 1 milligram of sulbactam per milliliter (estimated).

(iii) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (*T*) is satisfactory if it is not more than 1.5 at 10 percent of peak height in lieu of 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory for sulbactam if it is greater than 3,500 theoretical plates for a 30-centimeter column.

(C) *Resolution.* The resolution (*R*) between the peaks for sulbactam and penicillanic acid is satisfactory if it is not less than 3.8.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation ($S_R$ in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in §436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(B) of this section should not be changed.

(iv) *Calculations.* Calculate the micrograms of sulbactam per milligram of sample as follows:

$$\text{Micrograms of sulbactam per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

$A_u$=Area of the sulbactam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

$A_s$=Area of the sulbactam peak in the chromatogram of the sulbactam working standard;

$P_s$=Sulbactam activity in the sulbactam working standard solution in micrograms per milliliter;

$C_u$=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this chapter, using a solution containing 20 milligrams of sulbactam per milliliter.

(4) *Moisture.* Proceed as directed in §436.201 of this chapter.

(5) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

(6) *Identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the sulbactam working standard.

[52 FR 42290, Nov. 4, 1987; 52 FR 45281, Nov. 25, 1987, as amended at 54 FR 47205, Nov. 13, 1989; 55 FR 11585, Mar. 29, 1990]

### § 455.85 Vancomycin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin hydrochloride is the hydrochloride salt of a kind of vancomycin or a mixture of two or more such salts. It is soluble in water and moderately soluble in dilute methyl alcohol. It is insoluble in higher alcohols, acetone, and ether. It is so purified and dried that:

(i) It contains not less than 900 micrograms of vancomycin per milligram, calculated on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 5 percent.

(iv) Its pH in an aqueous solution containing 50 milligrams per milliliter is not less than 2.5 and not more than 4.5.

(v) It contains not more than 15 percent of factor A.

(vi) It gives a positive identity test for vancomycin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, factor A content, and identity.

(ii) Samples required: 12 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample of approximately 30 milligrams in sufficient sterile distilled water to give a stock solution of 1 milligram per milliliter (estimated). Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 10 micrograms of vancomycin per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using a solution containing 50 milligrams per milliliter.

(5) *Identity and factor A content.* Proceed as directed in §455.85a(b)(7).

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

### §455.85a Sterile vancomycin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile vancomycin hydrochloride is the hydrochloride salt of a kind of vancomycin or a mixture of two or more such salts. It is soluble in water and moderately soluble in dilute methyl alcohol. It is insoluble in higher alcohols, acetone, and ether. It is so purified and dried that:

(i) It contains not less than 900 micrograms of vancomycin per milligram, calculated on an anhydrous basis. If it is packaged for dispensing, its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of vancomycin that it is represented to contain.

(ii) It is sterile.

(iii) [Reserved]

(iv) It is nonpyrogenic.

(v) Its moisture content is not more than 5 percent.

(vi) Its pH in an aqueous solution containing 50 milligrams per milliliter is not less than 2.5 and not more than 4.5.

(vii) Its heavy metals content is not more than 30 parts per million.

(viii) It contains not more than 15 percent of factor A.

(ix) It gives a positive identity test for vancomycin.

(2) *Packaging.* In addition to the requirements of §432.1 of this chapter, if it is packaged for dispensing, the vancomycin content of each immediate container is 500 milligrams of vancomycin.

(3) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(4) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, heavy metals, factor A content, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use as an ingredient in the manufacture of another drug:

(*1*) For all tests except sterility: 12 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample of approximately 30 milligrams in sufficient sterile distilled water to give a stock solution of 1 milligram per milliliter; and also if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to give a stock solution of 1 milligram per milliliter. Further dilute an aliquot of the stock solution with solution 4 to the reference concentration of 10.0 micrograms of vancomycin per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use sterile distilled water in lieu of diluting fluid A.

(3) [Reserved]

(4) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams of vancomycin per milliliter.

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH*. Proceed as directed in § 436.202 of this chapter, using a solution containing 50 milligrams per milliliter.

(7) *Identity and factor A content*—(i) *Preparation of the chromatogram*—(*a*) *Equipment*. (*1*) Chromatographic paper (Whatman No. 1 untreated filter paper).

(*2*) Equipment for descending paper chromatography (Mitchell tank).

(*b*) *Preparations of solutions*—(*1*) *Factor A*. Prepare a solution in distilled water to contain 1.33 milligrams of factor A per milliliter and further dilute with distilled water to prepare solutions containing 0.1 and 0.2 milligram of factor A per milliliter.

(*2*) *Vancomycin working standard solution*. Prepare a solution in distilled water to contain 1.33 milligrams of vancomycin per milliliter.

(*3*) *Known mixture of factor A and vancomycin*. Prepare a solution in distilled water to contain 0.2 milligram of factor A and 1.13 milligrams of vancomycin (estimated) per milliliter.

(*4*) *Sample.* Prepare two solutions of the sample in distilled water, each to contain 1.33 milligrams of vancomycin (estimated) per milliliter.

(5) *Solvent mixture.* Mix 300 milliliters of butyl alcohol, 150 milliliters of pyridine, and 200 milliliters of water in a large separatory funnel and shake well for 3 minutes. Let stand at room temperature. There should be no separation of layers.

(*c*) *Procedure.* Saturate the atmosphere in the tank with vapors of the solvent mixture by placing 10 milliliters of the mixture in a trough in the bottom of the tank and closing tightly for 15 minutes. Prepare a sheet of chromatographic paper (8 inches x 8 inches) by carefully drawing a line of origin with a pencil 2 inches from one of the edges. Fold the paper along a straight line 1½ inches from the same edge of the paper. Starting 1 inch from the left-hand edge, establish points at 1-inch intervals along the line of origin on which to apply the solutions. Using a micropipette, apply the factor A solutions, the vancomycin solution, the known mixture solution, and the sample solutions by placing 5 microliters of each on separate spots. Properly identify the locations of the spots but avoid unnecessary handling of the paper. Allow the spots to dry spontaneously. Suspend the paper in the chamber so that the edge nearest the fold can be conveniently immersed in the solvent mixture contained in the top trough. Immerse the paper across its entire width to a depth sufficient to assure contact with the solvent mixture during the entire development time. Close the chamber tightly and allow the chromatograph to develop at room temperature for 6½ to 7 hours. Remove the paper and allow it to dry completely.

(ii) *Development by bioautograph*—(*a*) *Preparation of test organism* (*spore suspension*). The test organism is *Bacillus*

*subtilis* (ATCC 6633),[1] test organism H, prepared as described in § 436.103 of this chapter, using the method described in paragraph (b)(2) of that section.

(*b*) *Preparation of plates*—(*1*) *Baselayer.* Add 42 milliliters of medium 2 described in § 436.102(b)(2) of this chapter to each Petri dish (25 millimeters x 150 millimeters) and allow to harden on a flat, level surface. To prevent condensation of excess moisture, raise the tops slightly while the agar hardens.

(*2*) *Seed layer.* Melt nutrient agar medium 2 described in § 436.102(b)(2) of this chapter. Accurately measure a sufficient quantity of the melted agar, cool to 48° C., and add the appropriate quantity of the spore suspension prepared as described in paragraph (b)(7)(ii)(*a*) of this section. Swirl the flask of inoculated agar to obtain a homogeneous suspension. Add 8 milliliters of this inoculated agar to each plate, spread evenly, and allow to harden on a flat, level surface. For accurate results, it is necessary to obtain uniform distribution of the agar over the entire surface of the plates.

(*c*) *Assay.* For each spot on the paper described in paragraph (b)(7)(i)(*c*) of this section, cut a strip 1.5 centimeters by approximately 14 centimeters with the center of each strip centered about the line of descent of the spot. Place all strips on plates with the aid of forceps within as short a period of time as possible. Use maximum spacing between strips. Insure complete contact so that the entire strip becomes uniformly moistened. Allow to stand for 30 minutes. Remove the strips and identify each strip location on the Petri dish. Incubate the plates for 16–18 hours at 37° C. Any zone of inhibition corresponding to factor A in the sample must not be greater than that of the 0.2 milligram-per-milliliter factor A standard. Also, the two areas of inhibition for the sample due to the presence of factor A and vancomycin must compare to the corresponding two areas of inhibition of the known mixture in their respective distances from their origins.

[1] Available from: American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

(8) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

### § 455.86 Vancomycin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin is a tricyclic glycopeptide. It is a free flowing white to off-white colored powder. It is so purified and dried that:

(i) It contains not less than 925 micrograms of vancomycin per milligram, calculated on the anhydrous basis.

(ii) It contains not less than 92 percent vancomycin factor B and not more than 3 percent of any individual vancomycin related factor.

(iii) Its moisture content is not more than 20 percent.

(iv) Its heavy metals content is not more than 30 parts per million.

(v) It gives a positive identity test for vancomycin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, chromatographic purity, moisture, heavy metals, and identity.

(ii) Samples required: 12 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place an accurately weighed sample of approximately 100 milligrams in a 100-milliliter volumetric flask and dissolve in approximately 50 milliliters of distilled water and 1.0 milliliter of 0.1*N* hydrochloric acid. Swirl or sonicate to dissolve the sample and bring to volume with distilled water. Further dilute an aliquot of this solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 10 micrograms of vancomycin per milliliter (estimated).

(2) *Chromatographic purity.* Proceed as directed in § 436.366 of this chapter. The

relative amount of vancomycin B is not less than 92 percent, and the relative amount of any related substance is not more than 3 percent.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(5) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5 percent potassium bromide disc preparation as described in § 436.211(b)(1).

[59 FR 8400, Feb. 22, 1994]

### § 455.88 Rifabutin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rifabutin is an amorphous red-violet powder. It is (9*S*,12*E*,14*S*,15*R*,16*S*,17*R*,18*R*,19*R*,20*S*,21*S*,

22*E*,24*Z*)-

6,16,18,20-tetrahydroxy-1′-isobutyl-14-methoxy-

7,9,15,17,19,21,25-heptamethylspiro[9,4-(epoxypentadeca

[1,11,13]trienimino)-2*H*-furo[2′,3′:7,8] naphth[1,2-d]imidazole-2,4′-piperidine]-5,10,26-(3*H*,9*H*)-trione-16-acetate. It is very slightly soluble in water, sparingly soluble in ethanol, and soluble in chloroform and methanol. It is so purified and dried that:

(i) Its potency is not less than 950 micrograms and not more than 1,020 micrograms of rifabutin activity per milligram on an anhydrous basis.

(ii) Its content for the four major related substances detected by high-performance liquid chromatography (HPLC) is not more than 1.0 percent each. All other unknown related substances are not more than 0.5 percent. The total of all related substances is not more than 3.0 percent.

(iii) Its moisture content is not more than 2.5 percent.

(iv) Its *N*-isobutylpiperidone content is not more than 0.5 percent.

(v) It gives a positive identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for rifabutin potency, related substances, moisture, *N*-isobutylpiperidone, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 ± 1 nanometers, an 11 centimeters X 4.7 millimeters (i.d.) column packed with microparticulate (5 to 7 micrometers in diameter) packing material such as octylsilane chemically bonded to porous silica (U.S. Pharmacopeia designation L7), a flow rate of about 1.0 milliliter per minute, and a manual or automatic injector capable of injecting 10 microliters. The retention time for rifabutin is between 9 and 11 minutes. Reagents; working standard, sample, and resolution solutions; system suitability requirements; and calculations are as follows:

(i) *Reagents*—(A) *Hydrochloric acid, 2N.* Dilute 85 milliliters of hydrochloric acid (37 percent) with distilled water to 500 milliliters.

(B) *Potassium dihydrogen phosphate, 0.1M.* Prepare a solution containing 15.4 grams of potassium dihydrogen phosphate monohydrate (potassium phosphate monobasic) per liter of distilled water.

(C) *Sodium hydroxide, 2N.* Dissolve 8 grams of sodium hydroxide pellets in 100 milliliters of distilled water.

(D) *Mobile phase.* Acetonitrile:phosphate buffer, pH 6.5, 50:50. Mix equal quantities of acetonitrile and 0.1*M* potassium dihydrogen phosphate and adjust to an apparent pH of 6.5 ± 0.1 by dropwise addition of 2*N* sodium hydroxide. Filter through a suitable filter capable of removing particulate matter 0.5 micron in diameter and degas it just prior to its introduction into the chromatograph. Slight adjustments of the mobile phase components ratio may be made in order to meet the system suitability requirements described in the system suitability tests in paragraph (b)(1)(iii) of this section.

(ii) *Preparation of working standard, sample, and resolution test solution*—(A)

*Working standard solution*. Accurately weigh approximately 25 milligrams of the rifabutin working reference standard into a 50-milliliter volumetric flask. Add 5 milliliters of acetonitrile. Dissolve and dilute to volume with mobile phase and mix to obtain a solution having a known concentration of about 0.5 milligram of rifabutin per milliliter.

(B) *Sample solution*. Accurately weigh approximately 25 milligrams of sample into a 50-milliliter volumetric flask. Add 5 milliliters of acetonitrile. Dissolve and dilute to volume with mobile phase and mix to obtain a solution containing 0.5 milligram of rifabutin per milliliter (estimated).

(C) *Resolution test solution*. Dissolve approximately 10 milligrams of rifabutin in 2 milliliters of methanol and add 1 milliliter of 2*N* sodium hydroxide. Allow to stand for 3 to 4 minutes and then add 1 milliliter of 2*N* hydrochloric acid. Mix and dilute to 50 milliliters with mobile phase. Store aliquots of this solution in the frozen state for future use.

(iii) *System suitability requirements*. Using the apparatus and conditions described in this section, test the chromatographic system by injecting the resolution test solution. The chromatogram shows one major degradation peak and two minor degradation peaks eluting at relative retention times (RRT) of 0.5–0.6, 0.65–0.75, and 0.8–0.9, respectively, followed by the rifabutin peak.

(A) *Asymmetry factor*. The asymmetry factor ($A_S$) is satisfactory if it is not less than 1.0 and not more than 4.0 forther if a butin peak.

(B) *Efficiency of the column*. The absolute efficiency ($h_r$) is satisfactory if it is not more than 11 for the rifabutin peak, equivalent to 2,000 theoretical plates for a 11-centimeter column of 5-micrometer particles.

(C) *Resolution factor*. The resolution factor ($R$) between the peak for rifabutin and its closest eluting degradation product (generated in situ as described in paragraph (b)(1)(iii) of this section and eluting at RRT of 0.8–0.9) is satisfactory if it is not less than 1.3.

(D) *Coefficient of variation (relative standard deviation*). The coefficient of variation ($S_R$ in percent of 5 replicate injections of the rifabutin working standard solution) is satisfactory if it is not more than 2.0 percent. If the system suitability parameters have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations*. Calculate the micrograms of rifabutin per milligram of sample on an anhydrous basis as follows:

$$\text{Micrograms of rifabutin per milligram} = \frac{A_U \times P_s \times 100}{A_s \times C_U \times (100 - m)}$$

where:

$A_U$ = Area of the rifabutin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

$A_S$ = Area of the rifabutin peak in the chromatogram of the rifabutin working standard;

$P_S$ = Rifabutin activity in the rifabutin working standard solution in micrograms per milliliter;

$C_U$ = Milligrams of sample per milliliter of sample solution; and

m = Percent moisture content of the sample.

(2) *Related substances*. Proceed as directed in paragraph (b)(1) of this section for potency using the sample prepared as described in paragraph (b)(1)(ii)(B) of this section and calculating the amounts of related substances as follows.

(i) *Calculations*. Calculate the percentage of related substances as follows:

$$\text{Percent individual HPLC - related substance} = \frac{A_i \times 100}{A_t}$$

$$\text{Percent total HPLC - related substances} = \frac{A \times 100}{A_t}$$

where:

$A_i$ = Area of the individual related substance peak;

$A$ = The sum of areas of all peaks minus the area due to the rifabutin peak and solvent front peak; and

$A_t$ = The sum of areas of all peaks in the chromatogram excluding the solvent peak.

(ii) [Reserved]

(3) *Moisture*. Proceed as directed in § 436.201 of this chapter.

(4) *N-Isobutylpiperidone.* Proceed as directed in § 436.369 of this chapter.

(5) *Identity.* (i) Proceed as directed in § 436.211 of this chapter, using the sample preparation method described in paragraph (b)(1) of that section using a 1 to 2 percent mixture in potassium bromide.

(ii) The identity of rifabutin is confirmed by the qualitative comparison of the HPLC of the sample to the rifabutin working standard as directed in paragraph (b)(1) of this section.

[59 FR 40807, Aug. 10, 1994; 59 FR 46479, Sept. 8, 1994]

### § 455.90a Sterile vidarabine monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vidarabine monohydrate is the monohydrate form of 9-β - D - arabinofuranosyl - 9H - purin - 6-amine. It is a white to off-white powder. It is so purified and dried that:

(i) Its vidarabine content is not less than 845 micrograms and not more than 985 micrograms of vidarabine per milligram.

(ii) It is sterile.

(iii) [Reserved]

(iv) Its loss on drying is not less than 5 percent and not more than 7 percent.

(v) Its specific rotation in dimethylformamide at 25° C is —60.5°±4.5°.

(vi) It passes the identity test for vidarabine.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5(b) of this chapter, this drug shall be labeled ''vidarabine''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for vidarabine content, sterility, loss on drying, specific rotation, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 200 milligrams.

(b) *Tests and methods of assay*—(1) *Vidarabine content.* Proceed as directed in § 436.325 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use 100 milligrams in lieu of 300 milligrams.

(3) [Reserved]

(4) *Loss on drying.* Proceed as directed in § 436.200(e) of this chapter.

(5) *Specific rotation.* Using a solution containing 10 milligrams of vidarabine per milliliter in dimethylformamide and a polarimeter tube 1.0 decimeter in length, proceed as directed in § 436.210 of this chapter, except determine the specific rotation at 365 nanometers.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

[42 FR 44224, Sept. 2, 1977; 43 FR 9802, Mar. 10, 1978, as amended at 44 FR 30334, May 25, 1979; 50 FR 19921, May 13, 1985]

## Subpart B—Oral Dosage Forms

### § 455.110 Chloramphenicol capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol capsules are composed of chloramphenicol with or without one or more suitable and harmless diluents and lubricants. Each capsule contains 50, 100, or 250 milligrams of chloramphenicol. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of chloramphenicol that it is represented to contain. The chloramphenicol used conforms to the standards prescribed by § 455.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay; potency.* Use either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(1) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing 100 milliliters of 95 percent ethyl alcohol. Blend for 2 minutes. Then add 400 milliliters of distilled water and blend again for 2 minutes. Remove an aliquot and further dilute with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(2) *Spectrophotometric assay*—(i) *Preparation of working standard solution.* Dissolve approximately 50 milligrams of the working standard in 100 milliliters of distilled water. Warm if necessary to hasten dissolution. Transfer 10 milliliters into a 250-milliliter volumetric flask and fill to volume with distilled water.

(ii) *Procedure.* Place the contents of 10 capsules into a 250-milliliter volumetric flask. Add 50 milliliters of pure methyl alcohol to the flask and shake for at least 1 minute. Fill to volume with distilled water and mix thoroughly. Withdraw an aliquot and dilute with sufficient distilled water to give a concentration of 20 micrograms per milliliter. Using a suitable spectrophotometer equipped with a 1.0-centimeter cell and distilled water as the blank, determine the absorbance of the working standard and sample solutions at 278 nanometers. Calculate the potency as follows:

$$\text{Milligrams per capsule} = \frac{\text{Absorbance of sample} \times \text{labeled potency per capsule in milligrams}}{\text{Absorbance of standard}}$$

[39 FR 19149, May 30, 1974, as amended at 48 FR 3960, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

## § 455.111 Chloramphenicol palmitate oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol palmitate oral suspension is chloramphenicol palmitate and one or more suitable and harmless buffer substances, suspending agents, preservatives, colorings, and flavorings suspended in a suitable and harmless vehicle. Each milliliter contains chloramphenicol palmitate equivalent to 30.0 milligrams of chloramphenicol. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of chloramphenicol that it is represented to contain. Its pH is not less than 4.5 nor more than 7.0. Its content of polymorph A crystals does not exceed 10 percent. The chloramphenicol palmitate used conforms to the standards prescribed by § 455.11(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol palmitate used in making the batch for chloramphenicol content, melting range, specific rotation, and crystallinity.

(*b*) The batch for chloramphenicol content, pH, and content of polymorph A crystals.

(ii) Samples required:

(*a*) The chloramphenicol palmitate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) Chloramphenicol content (high-pressure liquid chromatography). Proceed as directed in § 436.335 of this chapter, except prepare the sample solution and calculate the chloramphenicol content as follows:

(i) *Preparation of sample solution.* Transfer a portion of the sample equivalent to 150 milligrams of chloramphenicol into a 200-milliliter volumetric flask. Add 100 milliliters of methanol and 4 milliliters of glacial

acetic acid. Shake and dilute to volume with methanol. Filter the solution through a glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter.

(ii) *Calculations.* Calculate the chloramphenicol content as follows:

$$\text{Milligrams of chloramphenicol per milliliter} = \frac{(A)(W_s)(f)(4)}{(B)(1{,}000)(V)}$$

where:

$A$=Area of the chloramphenicol palmitate sample peak (at a retention time equal to that observed for the standard);
$B$=Area of the working standard peak;
$W_s$=Weight of standard in milligrams;
$f$=Micrograms of chloramphenicol activity per milligram of chloramphenicol palmitate working standard; and
$V$=Volume of sample in milliliters.

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

(3) *Content of polymorph A crystal.*—(i) *Preparation of standards*—(*a*) *Standard containing 20 percent of polymorph A.* Prepare a thoroughly mixed, dry powder composed by weight of 1 part of polymorph A crystals of chloramphenicol palmitate and 4 parts of nonpolymorph A crystals of chloramphenicol palmitate.

(*b*) *Standard containing 10 percent of polymorph A.* Prepare a thoroughly mixed, dry powder composed by weight of 1 part of polymorph A crystals of chloramphenicol palmitate and 9 parts of nonpolymorph A crystals of chloramphenicol palmitate.

(ii) *Preparation of sample.* Place 20 milliliters of thoroughly mixed oral suspension into a 50-milliliter centrifuge tube. Add 20 milliliters of water and mix. Centrifuge for 10 to 15 minutes at a speed not less than 18,000 revolutions per minute. Decant the supernatant liquid. Wash the residue as follows: Add 2 milliliters of water to the residue, mix to make paste, add 18 milliliters of water, and mix thoroughly. Centrifuge, decant the supernatant liquid, and wash the residue two more times. Remove the washed residue from the centrifuge tube and dry it at least 14 hours in a vacuum desiccator at room temperature.

(iii) *Procedure.* Weigh 150 to 200 milligrams of liquid petrolatum into an agate mortar and add about 100 milligrams of standard or sample. Mix with a small spatula and then mull thoroughly with a pestle until a uniform consistency is obtained. Adjust a suitable infrared spectrophotometer so that 100 percent transmittance is recorded over the range of 11.0 to 13.0 microns. Use two rock salt plates as an absorption cell. Place a small drop of the mull in the center of one of the plates. Gently put the other plate on the mull and slowly squeeze the plates together to spread the mull uniformly. Clamp the two plates firmly together in a metal cell holder. Examine the assembled cell by holding it up to the light. It should appear smooth and free of any air bubbles and when placed in the instrument it should give a percent transmittance of 20 to 30 percent at 12.3 microns. Place the cell in the infrared spectrophotometer and record the absorption spectrum from 11.0 to 13.0 microns.

(iv) *Treatment of spectra*—(*a*) *Standard containing 20 percent of polymorph A.* Determine by inspection of the recorded spectrum the exact wavelengths of minimum absorption at approximately 11.3 and 12.65 microns. Also determine by inspection the exact wavelengths of maximum absorption at approximately 11.65 and 11.86 microns. In the following subdivision, references to these four nominal wavelengths are to the exact wavelengths observed on the particular instrument being used.

(*b*) *Standard containing 10 percent of polymorph A.* Draw a straight baseline between the minima occurring at 11.3 and 12.65 microns. Draw straight lines at 11.65 and 11.86 microns intersecting both the recorded spectrum and the baseline. Obtain the corrected absorbances at 11.65 and 11.86 microns and calculate the absorbance ratios as follows:

$$\text{Absorbance ratio} = \frac{{}^{S}11.65 - {}^{B}11.65}{{}^{S}11.86 - {}^{B}11.86}$$

where:

$^{S}11.65$=Absorbance value of recorded spectrum at 11.65 microns;
$^{B}11.65$=Absorbance value at point of intersection of the 11.65-micron line with the baseline;
$^{S}11.86$=Absorbance value of recorded spectrum at 11.86 microns;

$^{B}$11.86=Absorbance value at point of intersection of the 11.86-micron line with the baseline.

(*c*) *Sample.* Proceed as described in paragraph (b)(3)(iv)(*b*) of this section.

(v) *Calculation.* The absorbance ratio of the sample must be greater than the absorbance ratio of the standard containing 10 percent of polymorph A.

[39 FR 19166, May 30, 1974, as amended at 49 FR 6093, Feb. 17, 1984; 50 FR 19921, May 13, 1985]

### § 455.120 Cycloserine capsules.

(a) *Requirements for certification*—(1) *Standards of identity, quality, and purity.* Cycloserine capsules are capsules composed of crystalline cycloserine, with or without one or more suitable and harmless buffer substances, diluents, binders, and lubricants. Each capsule contains 250 milligrams of cycloserine. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of cycloserine that it is represented to contain. The loss on drying is not more than 1.0 percent. The cycloserine used conforms to the standards prescribed by § 455.20(a)(1).

(2) *Labeling.* In addition to the labeling prescribed by § 432.5 of this chapter, the labeling of each package shall bear a warning to the effect that the drug is to be used in patients with tuberculosis who fail to respond to treatment with isoniazid, streptomycin, paraaminosalicylic acid, viomycin, pyrazinamide, or combinations of these drugs, and that the drug may cause serious reactions such as convulsive seizures and mental disturbances.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Cycloserine used in making the batch for potency, loss on drying, pH, residue on ignition, crystallinity, and identity.

(*b*) The batch for cycloserine content and loss on drying.

(ii) Samples required:

(*a*) Cycloserine used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Using the cycloserine working standard as the standard of comparison, assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Chemical colorimetric assay*—(*a*) *Reagents.* (*1*) Acetic acid—1.0*N* solution.

(*2*) Sodium hydroxide—4.0*N* and 0.1*N* solutions.

(*3*) Sodium nitroprusside—4.0 percent solution: Dissolve 4.0 grams in sufficient distilled water to make 100.0 milliliters. Mix well. Store in amber bottle.

(*4*) Oxidized nitroprusside reagent—Mix equal parts of the 4.0 percent sodium nitroprusside solution and 4.0*N* sodium hydroxide, and let stand for 1 hour before using. Prepare daily and store in amber bottle.

(*5*) Cycloserine standard solution—dilute an appropriate-sized aliquot of the stock standard solution, prepared as directed in § 455.20(b)(1)(i)(*a*), in 0.1*N* sodium hydroxide to obtain a working standard solution containing 100 micrograms of cycloserine per milliliter.

(*b*) *Procedure.* Transfer the contents of 10 capsules into a 1,000-milliliter volumetric flask. Add 0.1*N* sodium hydroxide to dissolve the sample, and add sufficient 0.1*N* sodium hydroxide to measure 1,000 milliliters. Mix well and filter. Dilute an alliquot of the filtrate with sufficient 0.1*N* sodium hydroxide to give a concentration of 0.1 milligram per milliliter (estimated) and mix well. Pipette exactly 1.0 milliliter of the working standard solution and 1.0 milliliter of the sample solution into separate test tubes. Add exactly 3.0 milliliters of 1.0*N* acetic acid and exactly 1.0 milliliter of oxidized nitroprusside reagent to each of the test tubes; then mix thoroughly. Allow the tubes to stand at room temperature for 10 to 15 minutes, in order that maximum color intensity may develop. Using a reagent blank, determine the absorbance of the solutions at 625 nanometers in a suitable spectrophotometer.

Calculation:

$$\text{Milligrams of cycloserine per capsule} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \text{Labeled potency per capsule in milligrams}$$

(ii) *Microbiological turbidimetric assay.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules in a high-speed glass blender with sufficient sterile distilled water to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Further dilute the stock solution with sterile distilled water to the reference concentration of 50 micrograms of cycloserine per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

### §455.150 Calcium novobiocin oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium novobiocin oral suspension is a suspension containing calcium novobiocin and one or more suitable and harmless diluents, preservatives, suspending agents, surfactants, flavorings, and colorings in purified water. Each milliliter contains 25 milligrams of novobiocin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of novobiocin that it is represented to contain. The pH is not less than 6.0 and not more than 7.5. The calcium novobiocin used conforms to the standards prescribed by §455.50(a)(1) (i), (iv), (v), (vi), and (vii). If sodium novobiocin is reacted with a suitable calcium salt to form calcium novobiocin, the sodium novobiocin used conforms to the standards prescribed by §455.51(a)(1) (i), (iv), (v), (vi), (vii), and (viii).

(2) *Labeling.* It shall be labeled in accordance with §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The calcium novobiocin used in making the batch for potency, pH, crystallinity, identity, and specific rotation. If sodium novobiocin is used in making the batch: Potency, pH, residue on ignition, specific rotation, identity, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The calcium novobiocin or the sodium novobiocin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Remove a representative sample of the sirup with a suitable syringe and place into a high-speed glass blender with sufficient absolute ethyl alcohol to give a concentration (estimated) of 1,000 micrograms per milliliter. Blend for 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 1.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted suspension.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

### §455.151 Sodium novobiocin oral dosage forms.

### §455.151a Sodium novobiocin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin tablets are tablets that contain sodium novobiocin, with or without one or more suitable and harmless buffer substances, diluents, binders, and lubricants. Each tablet contains 125 milligrams or 250 milligrams of novobiocin. The 125-milligram tablet contains 375 milligrams of sulfamethizole. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of novobiocin that it is represented to contain. Its loss on drying is not more

than 3 percent. The tablets disintegrate within 1 hour. The sodium novobiocin used conforms to the standards prescribed by §455.51(a)(1).

(2) *Labeling.* It shall be labeled in accordance with §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Sodium novobiocin used in making the batch for potency, loss on drying, pH, residue on ignition, specific rotation, identity, and crystallinity.

(*b*) The batch for potency, loss on drying, disintegration time.

(ii) Samples required:

(*a*) Sodium novobiocin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Blend a representative number of tablets in a high-speed glass blender with sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in §436.212 of this chapter, using the method described in paragraph (e)(1) of that section.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

### §455.151b Sodium novobiocin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin capsules are gelatin capsules containing sodium novobiocin with a suitable and harmless filler and with or without a binder and a lubricant. Each capsule contains 100 milligrams or 250 milligrams of novobiocin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of novobiocin that it is represented to contain. The loss on drying is not more than 6.0 percent. The sodium novobiocin used conforms to the standards prescribed by §455.51(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sodium novobiocin used in making the batch for potency, loss on drying, pH, residue on ignition, specific rotation, crystallinity, and identity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The sodium novobiocin used in making the capsules: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules in a high-speed glass blender with 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

## §455.170 Rifampin oral dosage forms.

### §455.170a Rifampin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rifampin capsules are gelatin capsules containing rifampin with a suitable and harmless filler and with or without binders, lubricants, and stabilizers. Each sample contains 150 milligrams or 300 milligrams of rifampin. Its potency is satisfactory if it is not less than 90 percent and not more than

130 percent of the number of milligrams of rifampin that it is represented to contain. Its loss on drying is not more than 3.0 percent. The rifampin used conforms to the standards prescribed by § 455.70(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The rifampin used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The rifampin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing 200 milliliters of methyl alcohol and blend for 3 minutes. Add 300 milliliters of 1 percent potassium phosphate buffer, pH 6.0 (solution 1), and blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 5.0 micrograms of rifampin per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[39 FR 19166, May 30, 1974. Redesignated at 40 FR 53997, Nov. 20, 1975, and amended at 46 FR 46314, Sept. 18, 1981; 50 FR 19921, May 13, 1985]

### § 455.170b Rifampin-isoniazid capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rifampin-isoniazid capsules contain rifampin and isoniazid with a suitable and harmless filler and with or without binders, lubricants, and stabilizers in a gelatin capsule. Each capsule contains 300 milligrams of rifampin and 150 milligrams of isoniazid. Its rifampin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of rifampin that it is represented to contain. Its isoniazid content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of isoniazid that it is represented to contain. Its loss on drying is not more than 3.0 percent. The rifampin used conforms to the standards prescribed by § 455.70(a)(1). The isoniazid used conforms to the standards prescribed by the U.S.P.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The rifampin used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(*b*) The isoniazid used in making the batch for all U.S.P. specifications.

(*c*) The batch for rifampin content, isoniazid content, and loss on drying.

(ii) Samples required:

(*a*) The rifampin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 capsules.

(b) *Tests and methods of assay*—(1) *Rifampin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing 200 milliliters of methyl alcohol and blend for 3 minutes. Add 300 milliliters of 1 percent potassium phosphate buffer, pH 6.0 (solution 1), and blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 1 to the reference concentration of 5.0 micrograms of rifampin per milliliter (estimated).

(2) *Isoniazid content*—(i) *Equipment*—(*a*) *Electronic voltmeter.* A vacuum tube voltmeter or pH meter capable of measuring potentials from 0 to 1,400 millivolts.

(*b*) *Platinum electrodes.* Use twin platinum electrodes.

(*c*) *Constant current potential source.* Polarize the platinum electrodes by

means of a battery and a suitable resistance in series with the electrodes, or by a stable electronic power supply, so that the current flow is about 2.5 microamperes.

(*d*) *Titration vessel.* Use a 100-milliliter beaker.

(ii) *Reagents*—(*a*) Concentrated hydrochloric acid, reagent grade.

(*b*) 0.1*N* Bromine solution. Dissolve 3.0 grams of potassium bromate and 15.0 grams of potassium bromide in sufficient water to make 1 liter. Preserve in dark amber-colored, glass-stoppered bottles.

(*c*) 1.0*N* Potassium iodide. Dissolve 16.5 grams of potassium iodide in 100 milliliters of water.

(*d*) Starch iodide paste, T.S. (U.S.P.).

(*e*) 0.1*N* Sodium thiosulfate (U.S.P.).

(*f*) 0.1*N* Hydrochloric acid.

(*g*) Chloroform, reagent grade.

(iii) *Standardization of 0.1N bromine solution.* Measure accurately about 25 milliliters of the bromine solution into a 500-milliliter iodine flask and dilute with 120 milliliters of water. Add 5 milliliters of hydrochloric acid, insert the stopper in the flask, and shake it gently. Then add 5 milliliters of potassium iodide T.S., insert the stopper, shake the mixture, and allow it to stand for 5 minutes. Titrate the liberated iodine with standard 0.1*N* sodium thiosulfate U.S.P., adding starch iodide paste T.S./U.S.P. as the endpoint is approached. Calculate the normality of the bromine solution.

(iv) *Preparation of sample solution.* Empty the contents of not less than 10 capsules into a tared weighing bottle. Mix and weigh the powder. Calculate the average capsule weight content and accurately weigh a sample equivalent to approximately 100 milligrams of isoniazid. Transfer the sample to a 125-milliliter separatory funnel. Add 20 milliliters of 0.1*N* hydrochloric acid and shake well. Extract the acidic solution with six 25-milliliter portions of chloroform, combining any interfacial emulsion with the aqueous phase throughout the extraction procedure. Discard the chloroform extracts. Quantitatively transfer the acidic aqueous layer to a 100-milliliter volumetric flask and dilute to volume with 0.1*N* hydrochloric acid.

(v) *Titration procedure.* Pipet 25 milliliters of the sample solution into the titration vessel and add 10 milliliters of concentrated hydrochloric acid. Adjust the volume to approximately 50 milliliters with water. Titrate potentiometrically at constant current with 0.1*N* bromine solution to a dead stop endpoint. Calculate the isoniazid content for the sample used and determine the isoniazid content for the average capsule weight as follows:

$$\text{Milligrams isoniazid per average capsule} = \frac{V \times N \times 34.29 \times 4 \times W}{S}$$

where:

*V*=Volume in milliliters of 0.1*N* bromine solution used to titrate the sample;
*N*=Normality of bromine solution;
*W*=Average capsule weight content in milligrams;
*S*=Weight of sample in milligrams.

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

[40 FR 53997, Nov. 20, 1975, as amended at 50 FR 19921, May 13, 1985]

### §455.185 Vancomycin hydrochloride oral dosage forms.

### §455.185a Vancomycin hydrochloride for oral solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin hydrochloride for oral solution is vancomycin hydrochloride packaged in a suitable dispensing container. It may contain a suitable stabilizing agent. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of grams of vancomycin that it is represented to contain. Its moisture content is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 2.5 and not more than 4.5. The vancomycin hydrochloride used conforms to the standards prescribed by §455.85.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assay on:

(*a*) The vancomycin hydrochloride used in making the batch for potency, moisture, pH, factor A content, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The vancomycin hydrochloride used in making the batch: 12 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Empty the contents into an accurately measured volume of distilled water as directed in the labeling of the drug. Further dilute an aliquot with 0.1*M* passium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 10 micrograms of vancomycin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the drug reconstituted as directed in the labeling.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985. Redesignated at 51 FR 22072, June 18, 1986, and amended at 59 FR 8399, Feb. 22, 1994]

### § 455.185b Vancomycin hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin hydrochloride capsules contain vancomycin hydrochloride dispersed in polyethylene glycol. Each capsule contains either 125 milligrams or 250 milligrams of vancomycin. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of vancomycin that it is represented to contain. Its moisture is not more than 8 percent. It passes the dissolution test. The vancomycin hydrochloride used conforms to the standards prescribed by § 455.85(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The vancomycin hydrochloride used in making the batch for potency, moisture, pH, factor A content, and identity.

(*b*) The batch for potency, moisture, and dissolution.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The vancomycin hydrochloride used in making the batch: 12 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 100 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar with sufficient distilled water to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4) to the reference concentration of 10 micrograms of vancomycin per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the titration procedure described in paragraph (e)(1) of that section, except:

(i) Remove gelatin coating before grinding the capsules; and

(ii) Use solvent C in lieu of solvent A.

(3) *Dissolution.* Proceed as directed in § 436.215 of this chapter. The quantity *Q* (the amount of vancomycin dissolved) is 85 percent within 45 minutes.

[51 FR 22072, June 18, 1986, as amended at 55 FR 11585, Mar. 29, 1990]

### § 455.188 Rifabutin capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rifabutin capsules are gelatin capsules containing rifabutin with a suitable and harmless filler and with or without binders, lubricants, and stabilizers. Each capsule contains rifabutin equivalent to 150 milligrams of rifabutin. Its rifabutin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of rifabutin that it is represented to contain. Its

content of the four major related substances detected by high-performance liquid chromatography (HPLC) is not more than 1.0 percent each. All other unknown related substances are not more than 0.5 percent. The total of all related substances is not more than 4.5 percent. It passes the dissolution test if the quantity (Q) dissolved is 75 percent at 45 minutes. It passes the identity test. The rifabutin used conforms to the standards prescribed by § 455.88(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The rifabutin used in making the batch for potency, related substances, moisture, *N*-isobutylpiperidone, and identity.

(B) The batch for content, related substances, dissolution, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The rifabutin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Rifabutin content.* Proceed as directed in § 455.88(b)(1), preparing the sample solution and calculating the rifabutin content as follows:

(i) *Preparation of sample solution.* Empty 20 capsules, collecting the contents quantitatively. Weigh the powder and determine the average capsule fill weight. Mix the powder and accurately weigh a portion containing the equivalent of about 25 milligrams of rifabutin into a 50-milliliter volumetric flask. Add 5 milliliters of acetonitrile. Dilute to volume with mobile phase and mix to yield a solution containing 0.5 milligram of rifabutin per milliliter (estimated). Filter through a suitable filter capable of removing particulate matter 0.5 micron in diameter prior to injection into the chromatographic system.

(ii) *Calculations.* Calculate the rifabutin content as follows:

$$\text{Milligrams of rifabutin per capsule} = \frac{A_U \times C_S \times P_S \times W_a}{A_S \times C_U \times 1{,}000}$$

where:

$A_U$ = Area of the rifabutin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

$A_S$ = Area of the rifabutin peak in the chromatogram of the rifabutin working standard;

$C_S$ = Milligrams of rifabutin working standard per milliliter of standard solution;

$C_U$ = Milligrams of sample per milliliter of sample solution;

$P_S$ = Rifabutin activity in the rifabutin working standard solution in micrograms per milliliter; and

$W_a$ = Average capsule fill weight in milligrams.

(2) *Related substances.* Proceed as directed in paragraph (b)(1) of this section for rifabutin content using the sample prepared as described in paragraph (b)(1)(i) of this section and calculating the amounts of related substances as follows.

(i) *Calculations.* Calculate the percentage of related substances as follows:

$$\text{Percent individual HPLC-related substance} = \frac{A_i \times 100}{A_t}$$

$$\text{Percent total HPLC-related substances} = \frac{A \times 100}{A_t}$$

where:

$A_i$ = Area of the individual related substance peak;

$A$ = The sum of areas of all peaks minus the area due to the rifabutin peak and solvent front peak; and

$A_t$ = The sum of areas of all peaks in the chromatogram excluding the solvent peak.

(ii) [Reserved]

(3) *Dissolution test.* Proceed as directed in § 436.215 of this chapter. The quantity (Q) (the amount of rifabutin activity dissolved) is 75 percent within 45 minutes.

(4) *Identity.* (i) The retention time of the rifabutin response in the HPLC procedure described in paragraph (b)(1) of this section as applied to the sample

solution compares qualitatively to that of the rifabutin reference standard.

(ii) The identity of rifabutin capsules is also confirmed by the spectrophotometric identity test described in § 436.370 of this chapter.

[59 FR 40808, Aug. 10, 1994]

## Subpart C—Injectable Dosage Forms

### § 455.204 Aztreonam injectable dosage forms.

### § 455.204a Aztreonam for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Aztreonam for injection is a dry mixture of aztreonam and arginine. Its potency is satisfactory if each milligram of aztreonam for injection contains not less than 900 micrograms and not more than 1,050 micrograms of aztreonam when corrected for arginine content and moisture content. Its aztreonam immediate container fill (content) is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of aztreonam that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 2.0 percent. Its pH in an aqueous solution containing 100 milligrams of aztreonam per milliliter is not less than 4.5 and not more than 7.5. The aztreonam used conforms to the standards prescribed by § 455.4a(a)(1), except if the aztreonam for injection is manufactured by lyophilization, in which case the aztreonam need not be sterile.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The aztreonam used in making the batch for potency, sterility, pyrogens, moisture, residue on ignition, heavy metals, and identity. If the aztreonam for injection is made by lyophilization, the aztreonam need not be tested for sterility.

(*b*) The batch for aztreonam potency, aztreonam content, sterility, pyrogens. moisture. and pH.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The aztreonam used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency and content.* Determine both micrograms of aztreonam per milligram of sample and milligrams of aztreonam per container. Proceed as directed in § 436.361 of this chapter, except in addition to the column described in paragraph (a)(4) of that section, use a 5- to 50-centimeter saturator column having an inside diameter of 2 to 4.6 millimeters and packed with approximately 37 micrometer silica; and use the resolution test solution to determine resolution in lieu of the working standard solution. Perform the assay at ambient temperature, using an ultraviolet detection system operating at a wavelength of 206 nanometers, and a column packed with Chromegabond Diol (dihydroxypropane chemically bonded to porous silica), 5 to 10 micrometers or equivalent. Mobile phase, working standard solution, sample solution, resolution test solution, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Acetonitrile:0.01*M* ammonium phosphate, pH 2.0. Transfer 1.15 grams of ammonium phosphate monobasic to a 1-liter volumetric flask. Add about 800 milliliters of distilled water and sonicate to aid dissolution. Adjust the solution to pH 2.0 with o-phosphoric acid, 85 percent. Dilute the solution to volume with distilled water and mix well. Transfer about 250 milliliters of this solution and 750 milliliters of acetonitrile to a suitably sized container and mix well.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(*a*) *Working standard solution.* Transfer approximately 25 milligrams each of the aztreonam working standard and the

arginine working standard, accurately weighed, to a 25-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase (primary working standard solution). Further dilute with mobile phase to 0.2 milligram of aztreonam per milliliter (estimated).

(*b*) *Sample solutions.* Use separate containers for preparation of each sample solution as described in paragraph (b)(1)(ii)(*b*)(*1*) and (*2*) of this section.

(*1*) *Potency (micrograms of aztreonam per milligram).* Accurately weigh the container contents by difference and quantitatively transfer it to a 100-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase. Further dilute in mobile phase to 0.2 milligram of aztreonam per milliliter (estimated).

(*2*) *Content (milligrams of aztreonam per container).* If packaged in containers with capacities of less than 100 milliliters, reconstitute the sample as directed in the labeling, using distilled water in lieu of reconstituting fluid. If packaged in bottles with capacities of 100 milliliters or greater, reconstitute with 10 milliliters of distilled water. Withdraw the total contents of each container or bottle and dilute with mobile phase to a concentration of 0.2 milligram of aztreonam per milliliter (estimated).

(*c*) *Resolution test solution.* Dissolve 10 milligrams of open ring aztreonam, [[(2-amino-4-thiazolyl)[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-3-(sulfoamino)-butanoic acid, in 10.0 milliliters of primary standard solution. Further dilute 5 milliliters of this solution to 25.0 milliliters with mobile phase.

(iii) *System suitability requirements*—(*a*) *Tailing factor.* The tailing factor (*T*) of the aztreonam peak is satisfactory if it is not more than 2 at 5 percent of peak height.

(*b*) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,000 theoretical plates.

(*c*) *Resolution.* The resolution (*R*) between aztreonam peak and open ring aztreonam is satisfactory if it is not less than 2.0.

(*d*) *Coefficient of variation.* The coefficient of variation ($S_R$in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in §436.361(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(*b*) of this section should not be changed.

(iv) *Calculations*—(*a*) *Potency (micrograms per milligram).* (*1*) Calculate the micrograms of aztreonam per milligram (uncorrected) as follows:

$$\text{Micrograms of aztreonam per milligram (uncorrected)} = \frac{A_u \times P_s}{A_s \times C_u}$$

where:

$A_u$=Area of the aztreonam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

$A_s$=Area of the aztreonam peak in the chromatogram of the working standard;

$P_s$=Aztreonam activity in the working standard solution in micrograms per milliliter; and

$C_u$=Milligrams of sample per milliliter of sample solution.

(*2*) Calculate the micrograms of arginine per milligram as follows:

$$\text{Micrograms of arginine per milligram} = \frac{A_u \times P_s}{A_s \times C_u}$$

where:

$A_u$=Area of the arginine peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

$A_s$=Area of the arginine peak in the chromatogram of the working standard;

$P_s$=Arginine activity in the working standard solution in micrograms per milliliter; and

$C_u$=Milligrams of sample per milliliter of sample solution.

(*3*) Calculate the micrograms of aztreonam per milligram (corrected) as follows:

$$\text{Micrograms of aztreonam per milligram (corrected)} = \frac{\text{Micrograms of aztreonam per milligram (uncorrected)} \times 1{,}000}{1{,}000 - [\text{Micrograms of arginine per milligram} + (\text{Percent moisture}) \times 10]}$$

(*b*) *Content (milligrams of aztreonam per container).* Calculate the aztreonam content of the container as follows:

$$\text{Milligrams of aztreonam per container} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

$A_u$=Area of the aztreonam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

$A_s$=Area of the aztreonam peak in the chromatogram of the working standard;

$P_s$=Aztreonam activity in the aztreonam working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of aztreonam per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams of aztreonam per milliliter.

[52 FR 4615, Feb. 13, 1987; 52 FR 8550, Mar. 18, 1987. Redesignated at 54 FR 40385, Oct. 2, 1989, and amended at 54 FR 41824, Oct. 12, 1989; 55 FR 11585, Mar. 29, 1990]

### § 455.204b Aztreonam injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Aztreonam injection is a frozen aqueous iso-osmotic solution of aztreonam and arginine. Each milliliter contains aztreonam equivalent to either 10 milligrams, 20 milligrams, or 40 milligrams. Its aztreonam content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of aztreonam that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 4.5 and not more than 7.5. It passes the identity test. The aztreonam used conforms to the standards prescribed by § 455.4(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The aztreonam used in making the batch for potency, moisture, residue on ignition, heavy metals, and identity.

(B) The batch for aztreonam potency, sterility, pyrogens, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The aztreonam used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Potency.* Proceed as directed in § 436.361 of this chapter, except in addition to the column described in paragraph (a)(4) of that section, use a 5- to 50-centimeter saturator column having an inside diameter of 2 to 4.6 millimeters and packed with approximately 37 micrometer silica, and use the resolution test solution to determine resolution in lieu of the working standard solution. Perform the assay at ambient temperature, using an ultraviolet detection system operating a wavelength of 206 nanometers, and a column packed with Chromegabond Diol (dihydroxypropane chemically bonded to porous silica), 5 to 10 micrometers or equivalent. Mobile phase, working

standard solution, sample solution, resolution test solution, system suitability requirements, and calculations as follows:

(i) *Mobile phase.* Acetonitrile: 0.01*M* pH 2.0 ammonium phosphate (75:25). Transfer 1.15 grams of ammonium phosphate monobasic to a 1-liter volumetric flask. Add about 800 milliliters of distilled water and sonicate to aid dissolution. Adjust the solution to pH 2.0 with *o*-phosphoric acid, 85 percent. Dilute the solution to volume with distilled water and mix well. Transfer about 250 milliliters of this solution and 750 milliliters of acetonitrile to a suitable-sized container and mix well. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution.* Transfer approximately 25 milligrams each of the aztreonam working standard and the arginine working standard, accurately weighed, to a 25-milliliter volumetric flask. Dissolve and dilute to volume with mobile phase (primary working standard solution). Further dilute with mobile phase to obtain a solution containing 0.2 milligram of aztreonam per milliliter.

(B) *Sample solution.* Using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container and dilute with sufficient mobile phase to obtain a solution containing 0.2 milligram of aztreonam per milliliter (estimated).

(C) *Resolution test solution.* Dissolve 10 milligrams of open ring aztreonam, 2-[[(2-amino-4-thiazolyl)[(1-carboxy-1-methylethoxy)imino]acetyl]amino]-3-(sulfoamino)-butanoic acid, in 10.0 milliliters of primary standard solution. Further dilute 5 milliliters of this solution to 25.0 milliliters with mobile phase.

(iii) *System suitability requirements*—(A) *Tailing factor.* The tailing factor (*T*) of the aztreonam peak is satisfactory if it is not more than 2 at 5 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (*n*) is satisfactory if it is greater than 1,000 theoretical plates.

(C) *Resolution.* The resolution (*R*) between the aztreonam peak and open ring aztreonam is satisfactory if it is not less than 2.0.

(D) *Coefficient of variation.* The coefficient of variation ($S_R$ in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

If the system suitability requirements have been met, then proceed as described in §436.361(b) of this chapter. Alternative chromatographic conditions are acceptable, provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(B) of this section should not be changed.

(iv) *Calculations:* Calculate the milligrams of aztreonam per milliliter of sample as follows:

$$\text{Milligrams of aztreonam per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

$A_u$=Area of the aztreonam peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

$A_s$=Area of the aztreonam peak in the chromatogram of the working standard;

$P_s$=Aztreonam activity in the aztreonam working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this chapter, except inject a sufficient volume of the undiluted solution to deliver 50 milligrams of aztreonam per kilogram.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted solution.

(5) *Identity.* The high-performance liquid chromatogram of the sample is determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the aztreonam working standard.

[54 FR 40385, Oct. 2, 1989]

### § 455.210 Chloramphenicol injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol injection is chloramphenicol, with or without one or more suitable and harmless buffer substances, dissolved in one or more suitable and harmless solvents. Each milliliter contains 250 milligrams of chloramphenicol. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of chloramphenicol that it is represented to contain. It is sterile. It is nonpyrogenic. Its pH is not less than 4.7 and not more than 5.0. The chloramphenicol used conforms to the standards prescribed by § 455.10a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, and pH.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of eight immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample in sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except add the contents of each container directly to the dry filter, thus eliminating the preliminary solubilization step.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 5 milligrams per milliliter.

(4)—(5) [Reserved]

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted drug.

[39 FR 19166, May 30, 1974, as amended at 45 FR 64568, Sept. 30, 1980; 48 FR 3961, Jan. 28, 1983; 48 FR 7440, Feb. 22, 1983; 50 FR 19921, May 13, 1985]

### § 455.212 Sterile chloramphenicol sodium succinate.

The requirements for certification and the tests and methods of assay for sterile chloramphenicol sodium succinate packaged for dispensing are described in § 455.12a.

[43 FR 9801, Mar. 10, 1978]

### § 455.230 Moxalactam disodium for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Moxalactam disodium for injection is a dry mixture of moxalactam disodium and mannitol. Its moxalactam content is satisfactory if it is not less than 90 percent and not more tha 120 percent of the number of milligrams of moxalactam that it is represented to contain. The moxalactam content of the dry mixture is not less than 722 micrograms of moxalactam per milligram. The ratio of R-isomer to S-isomer is not less than 0.8 and not more than 1.4. It is sterile. It is nonpyrogenic. Its moisture content is not more than 3.0 percent. Its pH is not less than 4.5 and not more than 7.0. It passes the identity test for moxalactam.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for moxalactam content, isomer

ratio, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples required on the batch:

(*a*) For all tests except sterility: A minimum of 10 immediate containers.

(*b*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Moxalactam content; isomer ratio.* Proceed as directed in § 436.332 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 50 milligrams of moxalactam.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

(7) *Identity.* Proceed as directed in § 436.333 of this chapter.

[46 FR 61070, Dec. 15, 1981, as amended at 50 FR 19921, May 13, 1985]

### § 455.251 Sodium novobiocin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin for injection is sodium novobiocin with or without one or more suitable solubilizing agents, preservatives, and diluents. Each vial contains 500 milligrams of novobiocin. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of novobiocin that it is represented to contain. It is sterile and nonpyrogenic. Its loss on drying is not more than 6.0 percent. Its pH, when reconstituted as directed in the labeling, is not less than 6.5 and not more than 8.5. The sodium novobiocin used conforms to the standards prescribed by § 455.51a(a)(1) (i), (iii), (v), (vi), (vii), and (viii).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The sodium novobiocin used in making the batch for potency, loss on drying, pH, residue on ignition, specific rotation, crystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(*a*) The sodium novobiocin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32 of this chapter, using a solution containing 10 milligrams of novobiocin per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the sample after

reconstituting as directed in the labeling.

[39 FR 19166, May 20, 1974, as amended at 46 FR 25608, May 8, 1981; 50 FR 19921, May 13, 1985]

### § 455.270 Rifampin for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rifampin for injection is a dry mixture of rifampin, sodium formaldehyde sulfoxylate, and sodium hydroxide. Its potency is 600 milligrams per vial. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of rifampin that it is represented to contain. It is sterile. It is nonpyrogenic. Its moisture content is not more than 3.0 percent. Its pH is not less than 7.8 and not more than 8.8. It passes the identity test. The rifampin used conforms to the standards prescribed by § 455.70(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The rifampin used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(B) The batch for potency, sterility, pyrogens, moisture, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research.

(A) The rifampin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove the withdrawable contents from each container represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the preparation, withdraw an accurately measured volume from each container. Dilute with 1 percent potassium phosphate buffer, pH 6.0 (solution 1) to give a stock solution of 1.0 milligram of rifampin per milliliter (estimated). Further dilute an aliquot of the stock solution with solution 1 to the reference concentration of 5.0 micrograms of rifampin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 10 milligrams of rifampin per milliliter.

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using a concentration of 60 milligrams of rifampin per milliliter.

(6) *Identity.* Proceed as directed in § 436.365 of this chapter.

[54 FR 38375, Sept. 18, 1989]

### § 455.280a Sterile spectinomycin hydrochloride.

The requirements for certification and the tests and methods of assay for sterile spectinomycin hydrochloride packaged for dispensing are described in § 455.80a.

### § 455.285 Vancomycin hydrochloride injectable dosage forms.

### § 455.285a Sterile vancomycin hydrochloride.

The requirements for certification and the tests and methods of assay for sterile vancomycin hydrochloride packaged for dispensing are described in § 455.85a.

### § 455.285b Vancomycin hydrochloride for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin hydrochloride for injection is a dry mixture of vancomycin hydrochloride and a suitable stabilizing agent. It contains not less than 925 micrograms of vancomycin per milligram, calculated

on an anhydrous basis. Its vancomycin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of vancomycin that it is represented to contain. It contains not less than 88 percent vancomycin factor B. It contains not more than 4 percent of any individual vancomycin related factor. It is sterile. It is nonpyrogenic. Its moisture content is not more than 5 percent. The pH of an aqueous solution containing 50 milligrams per milliliter is not less than 2.5 and not more than 4.5. Its heavy metals content is not more than 30 parts per million. It gives a positive identity test. The vancomycin hydrochloride used conforms to the standards prescribed by §455.85(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Request for certification; samples.* In addition to the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The vancomycin hydrochloride used in making the batch for potency, moisture, pH, factor A content, and identity.

(B) The batch for vancomycin potency, vancomycin content, chromatographic purity, sterility, pyrogens, moisture, pH, heavy metals, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The vancomycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Test and methods of assay*—(1) *Vancomycin potency and content.* Determine both micrograms of vancomycin per milligram of sample and milligrams of vancomycin per container. Proceed as directed in §435.105 of this chapter, preparing the sample solution as follows:

(i) *Preparation of sample solution.* Use separate containers for preparation of each sample solution as described in paragraphs (b)(1)(i) (A) and (B) of this section.

(A) *Micrograms of vancomycin per milligram.* Dissolve an accurately weighed sample of approximately 30 milligrams in sufficient distilled water to obtain a stock solution of 1 milligram per milliliter. Further dilute an aliquot of the stock solution with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4) to the reference concentration of 10.0 micrograms of vancomycin per milliliter (estimated).

(B) *Milligrams of vancomycin per container.* Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of vancomycin content in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4) to the reference concentration of 10.0 micrograms of vancomycin per milliliter (estimated).

(2) *Chromatographic purity.* Proceed as directed in §436.366 of this chapter. The relative amount of vancomycin B is not less than 88 percent and the relative amount of any related substance is not more than 4 percent.

(3) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use sterile distilled water in lieu of diluting fluid A.

(4) *Pyrogens.* Proceed as directed in §436.32(a) of this chapter, using a solution containing 5 milligrams of vancomycin per milliliter.

(5) *Moisture.* Proceed as directed in §436.201 of this chapter.

(6) *pH.* Proceed as directed in §436.202 of this chapter, using a solution containing 50 milligrams per milliliter.

(7) *Heavy metals.* Proceed as directed in §436.208 of this chapter.

(8) *Identity.* Proceed as directed in §436.211 of this chapter, using the 0.5 percent potassium bromide disc preparation as described in paragraph (b)(1) of that section.

[54 FR 20384, May 11, 1989; 54 FR 22838, May 28, 1989]

**§ 455.285c Vancomycin hydrochloride injection.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin hydrochloride injection is a frozen, aqueous, iso-osmotic solution of vancomycin hydrochloride and a tonicity adjusting agent. Each milliliter contains vancomycin hydrochloride equivalent to 5 milligrams of vancomycin. Its vancomycin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of vancomycin that it is represented to contain. It contains not less than 88 percent vancomycin factor B. It contains not more than 4 percent of any individual vancomycin related factor. It is sterile. It contains not more than 0.33 U.S.P. Endotoxin Unit per milligram of vancomycin hydrochloride. Its pH is not less than 3.0 and not more than 5.0. The vancomycin used conforms to the standards prescribed by § 455.86.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter. In addition, this drug shall be labeled ''vancomycin hydrochloride injection.''

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The vancomycin used in making the batch for vancomycin potency, chromatographic purity, moisture, heavy metals, and identity.

(B) The batch for vancomycin content, chromatographic purity, sterility, bacterial endotoxins, pH, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The vancomycin used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 12 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay.* Thaw the sample as directed in the labeling. The sample solution used for testing must be at room temperature.

(1) *Vancomycin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample solution as follows: Using a suitable hypodermic needle and syringe, remove an accurately measured representative portion from each container immediately after thawing and reaching room temperature. Dilute with 0.1*M* potassium phosphate buffer, pH 4.5 (solution 4), to the reference concentration of 10 micrograms of vancomycin per milliliter (estimated).

(2) *Chromatographic purity.* Proceed as directed in § 436.366 of this chapter. The relative amount of vancomycin B is not less than 88 percent and the relative amount of any related substance is not more than 4 percent.

(3) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in § 436.20(e)(1), except use sterile distilled water in lieu of diluting fluid A.

(4) *Bacterial endotoxins.* Proceed as directed in the U.S.P. bacterial endotoxins test. The specimen under test contains not more than 0.33 U.S.P. Endotoxin Unit per milligram of vancomycin hydrochloride.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

(6) *Identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(2) of this section compares qualitatively to that of the vancomycin working standard.

[59 FR 8400, Feb. 22, 1994]

**§ 455.290 Vidarabine monohydrate for infusion.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vidarabine monohydrate for infusion contains in each milliliter vidarabine monohydrate equivalent to 187.4 milligrams of vidarabine in an aqueous suspension containing suitable and harmless buffers and preservatives. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of vidarabine that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no histamine or histamine-like substances. Its pH is

not less than 5.0 and not more than 6.2. The vidarabine monohydrate used conforms to the standards prescribed by §455.90a (a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by §432.5 of this chapter, this drug shall be labeled ''vidarabine for infusion''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The vidarabine monohydrate used in making the batch for vidarabine content, loss on drying, specific rotation, and identity.

(*b*) The batch for vidarabine content, sterility, pyrogens, histamine, and pH.

(ii) Samples required:

(*a*) The vidarabine monohydrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 16 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Vidarabine content.* Proceed as directed in §436.325 of this chapter, except prepare the sample solution and calculate the vidarabine content as follows:

(i) *Preparation of sample solution.* Using a suitable hypodermic needle and syringe, transfer 2 milliliters of the well-shaken suspension to a 500-milliliter volumetric flask. Add approximately 50 milliliters of distilled water and 5 milliliters of glacial acetic acid. Warm on a steam bath for 15 minutes to dissolve the vidarabine. Cool to room temperature and dilute to volume with distilled water. Transfer 4 milliliters to a 25-milliliter volumetric flask and dilute to volume with distilled water.

(ii) *Calculations.* Calculate the vidarabine content as follows:

$$\text{Milligrams of vidarabine per milliliter} = \frac{A \times W_s \times f \times 125}{B \times 1{,}000 \times 16,}$$

where:

$A$=Area of the vidarabine sample peak (at a retention time equal to that observed for the standard);

$B$=Area of the standard peak;

$W_s$=Weight of the standard in milligrams; and

$f$=Potency of standard in micrograms per milligram.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(2) of that section.

(3) *Pyrogens.* Proceed as directed in §436.32(a) of this chapter, using a solution containing 10 milligrams of vidarabine per milliliter.

(4) *Histamine.* Proceed as directed in §436.35 of this chapter. Apply sufficient heat to dissolve the vidarabine.

(5) *pH.* Proceed as directed in §436.202 of this chapter, using the undiluted suspension.

[44 FR 1374, Jan. 5, 1979, as amended at 44 FR 30334, May 25, 1979; 50 FR 19921, May 13, 1985]

## Subpart D—Ophthalmic Dosage Forms

### §455.310 Chloramphenicol ophthalmic dosage forms.

### §455.310a Chloramphenicol ophthalmic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol ophthalmic solution contains in each milliliter 5 milligrams of chloramphenicol with or without one or more suitable and harmless preservatives, buffer substances, and surfactants, in an aqueous solution. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of chloramphenicol that it is represented to contain. It is sterile. Its pH is not less than 3 nor more than 6; however, if the solution is buffered, its pH is not less than 7.0 nor more than 7.5. The chloramphenicol used conforms to the standards prescribed by §455.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 containers, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH*. Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

[39 FR 19166, May 30,1974, as amended at 43 FR 59057, Dec. 19, 1978; 46 FR 46313, Sept. 18, 1981; 48 FR 3961, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

### § 455.310b Chloramphenicol for ophthalmic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Chloramphenicol for ophthalmic solution contains 25 milligrams of chloramphenicol with one or more suitable and harmless buffer substances. When reconstituted as directed in the labeling, its potency is not less than 90 percent and not more than 130 percent of the number of milligrams of chloramphenicol that it is represented to contain. It is sterile. Its pH is not less than 7.1 and not more than 7.5. The chloramphenicol used conforms to the standards prescribed by § 455.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods:

(i) *Microbiological turbidimetric assay*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an accurately measured representative aliquot of the sample with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *Spectrophotometric assay*. Reconstitute the sample as directed in the labeling and dilute a 1.0-milliliter aliquot in sufficient distilled water to obtain a solution containing 20 micrograms of chloramphenicol per milliliter. Dissolve an accurately weighed portion of the working standard in sufficient distilled water to obtain a solution containing 20 micrograms per milliliter. Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of the sample and standard solutions at 278 nanometers.

Calculate the potency of the sample as follows:

$$\text{Milligrams of chloramphenicol per milliliter} = \frac{\text{Absorbance of sample} \times \text{labeled potency per milliliter in milligrams}}{\text{Absorbance of standard}}$$

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 5 milligrams per milliliter.

[49 FR 6093, Feb. 17, 1984, as amended at 50 FR 19921, May 13, 1985]

### §455.310c Chloramphenicol ointment (chloramphenicol cream).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol ointment is chloramphenicol in a suitable and harmless ointment base, with or without suitable and harmless buffer substances, dispersing and suspending agents. It may contain cortisone or a suitable derivative of cortisone. If such base is water-miscible, it shall contain a suitable and harmless preservative. Its potency is not less than 1.0 milligram per gram. If it is intended for ophthalmic use, it is sterile. The chloramphenicol used conforms to the requirements of §455.10a(a)(1), except paragraphs (a)(1) (ii), (iii), and (v) of that section. The chloramphenicol used in making the chloramphenicol ophthalmic ointment conforms to the requirements of §455.10a(a)(1), except paragraphs (a)(1) (iii) and (v) of that section. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Packaging.* Unless it is packaged in a single dose container, chloramphenicol ointment shall be packaged in collapsible tubes, which shall be well-closed containers as defined by the U.S.P., and shall not be larger than the ⅛-ounce size if such ointment is represented for ophthalmic use, and in no case larger than the 2-ounce size, except that if it is labeled solely for hospital use it may be packaged in immediate containers of glass which meet the test for tight containers as defined by the U.S.P. The composition of the immediate container and closure shall be such as will not cause any change in the strength, quality, or purity of the contents beyond any limit therefor in applicable standards, except that minor changes so caused which are normal and unavoidable in good packaging, storage, and distribution practice shall be disregarded.

(3) *Labeling.* In addition to the labeling requirements prescribed by §201.100 of this chapter (regulations issued under section 502(f) of the act), each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the outside wrapper or container and the immediate container the statement ''Expiration date _____'', the blank being filled in with the date that is 60 months, or 24 months if it is packaged in an immediate container other than tin or glass, or 12 months if the ointment base is water miscible, after the month during which the batch was certified.

(ii) If it contains one of the active ingredients specified in paragraph (a)(1) of this section, after the name ''chloramphenicol ointment'', wherever it appears, the name of the active ingredient, in juxtaposition with such name.

(4) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting point, and absorptivity.

(*b*) The batch for potency and for sterility if the ointment is intended for ophthalmic use.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 5 immediate containers if it is packaged in immediate containers of tin or glass; a minimum of 20 immediate containers if it is packaged in immediate containers other than tin or glass.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows:

(i) *If the ointment is water miscible.* Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient distilled water to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *If the ointment is not water miscible.* Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of petroleum ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of distilled water and shake well. Allow the layers to separate. Remove the aqueous layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of distilled water. Combine the aqueous extractives in a suitable volumetric flask and dilute to volume with distilled water. Remove an aliquot and further dilute with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated). The potency of chloramphenicol ointment is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of milligrams of chloramphenicol that it is represented to contain.

(2) *Sterility.* If the ointment is intended for ophthalmic use, proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section. However, if the ointment is not soluble in isopropyl myristate proceed as directed in § 436.20 of this chapter, using the method described in § 436.20(e)(2), except use 100 milligrams in lieu of 300 milligrams of solids.

[39 FR 19166, May 30, 1974, as amended at 41 FR 10886, Mar. 15, 1976; 44 FR 10380, Feb. 20, 1979; 48 FR 3961, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

### § 455.310d Chloramphenicol-polymyxin ointment.

(a) *Requirements for certification.* Chloramphenicol-polymyxin ointment conforms to all requirements and is subject to all procedures prescribed by § 455.310c(a) for chloramphenicol ointment, except that:

(1) It contains not less than 10,000 units of polymyxin B per gram. The polymyxin B used conforms to the requirements prescribed for polymyxin B by § 444.170a(a)(1) of this chapter.

(2) In lieu of the labeling prescribed by § 455.310c(a)(3)(i)(*a*), each package shall bear on the outside wrapper or container and the immediate container, the statement ''Expiration date ————'', the blank being filled in with the date that is 24 months after the month during which the batch was certified, except that the blank may be filled in with the date that is 36 months, 48 months, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that after having been stored for such period of time such drug as prepared by him complies with the standards prescribed by this section: *Provided however,* That such expiration date may be omitted from the immediate container if it contains a single dose and it is packaged in an individual wrapper or container.

(3) In addition to complying with the requirements of § 455.310c(a)(4), a person who requests certification of a batch

shall submit with his request a statement showing the batch mark and (unless previously submitted) the results and date of the latest tests and assays of the polymyxin used in making the batch for potency. He shall also submit in connection with his request a sample consisting of not less than 6 packages of ointment and (unless it was previously submitted) a sample consisting of 5 packages containing approximately equal portions of not less than 0.5 gram each of the polymyxin used in making the batch.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Chloramphenicol content.* Proceed as directed in §455.310c(b). Its chloramphenicol content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams per gram that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in §444.170a(b)(2)(i) of this chapter, except in lieu of the directions in §444.170a(b)(2)(i)(*g*) of this chapter for the preparation of the sample, prepare the sample as follows: Place an accurately weighed sample (usually approximately 1.0 gram) in a separatory funnel containing approximately 50 milliliters of peroxide-free ether, and shake the sample and ether until homogeneous. Add 25 milliliters of 10-percent potassium phosphate buffer, pH 6.0 and shake. Remove the buffer layer and repeat the extraction with three additional 25-milliliter portions of buffer. Combine the extractives and make the proper estimated dilutions, using the buffer solution, except that, if the sample contains a water-soluble base, place an accurately weighed representative sample in a blending jar containing 1.0 milliliter of polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0, to give a final volume of 200 milliliters. Using a highspeed blender, blend the mixture for 2 minutes to 3 minutes and then make the proper estimated dilutions with 10 percent phosphate buffer pH 6.0. Its content of polymyxin is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of units per gram that it is represented to contain.

(2) *Sterility.* If the ointment is intended for ophthalmic use, proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(3) of that section. However, if the ointment is not soluble in isopropyl myristate proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use 100 milligrams in lieu of 300 milligrams of solids.

[39 FR 19166, May 30, 1974, as amended at 47 FR 23443, May 28, 1982; 50 FR 19921, May 13, 1985]

### §455.310e Chloramphenicol-hydrocortisone acetate for ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol-hydrocortisone acetate for ophthalmic suspension contains 12.5 milligrams of chloramphenicol and 25 milligrams of hydrocortisone acetate with one or more suitable and harmless buffer substances, preservatives, and diluents. When reconstituted as directed in the labeling, its potency is not less than 90 percent and not more than 130 percent of the number of milligrams of chloramphenicol that it is represented to contain. It is sterile. Its pH is not less than 7.1 and not more than 7.5. The chloramphenicol used conforms to the standards prescribed by §455.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity.

(*b*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Use either of the following methods:

(i) *Microbiological turbidimetric assay*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Dilute an accurately measured representative aliquot of the sample with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(ii) *Spectrophotometric assay*. Reconstitute the sample as directed in the labeling and dilute a 1.0-milliliter aliquot in sufficient distilled water to obtain a solution containing 20 micrograms of chloramphenicol per milliliter. Dissolve an accurately weighed portion of the working standard in sufficient distilled water to obtain a solution containing 20 micrograms per milliliter. Using a suitable spectrophotometer and distilled water as the blank, determine the absorbance of the sample and standard solutions at 278 nanometers. Calculate the potency of the sample as follows:

Milligrams of chloramphenicol per milliliter=Absorbance of sample X labeled potency per milliliter in milligrams/Absorbance of standard.

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH*. Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 5 milligrams per milliliter.

[49 FR 6093, Feb. 17, 1984]

### § 455.390 Vidarabine monohydrate ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Vidarabine monohydrate ophthalmic ointment contains in each gram vidarabine monohydrate equivalent to 28.11 milligrams of vidarabine in a suitable and harmless base. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of vidarabine that it is represented to contain. It is sterile. It passes the test for metal particles. The vidarabine monohydrate used conforms to the standards prescribed by § 455.90a(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5 of this chapter, this drug shall be labeled ''vidarabine ophthalmic ointment''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:k

(*a*) The vidarabine monohydrate used in making the batch for vidarabine content sterility, loss on drying, specific rotation, and identity.

(*b*) The batch for vidarabine content, sterility, and metal particles.

(ii) Samples required:

(*a*) The vidarabine monohydrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 16 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(*b*) *Tests and methods of assay*—(1) *Vidarabine content.* Proceed as directed in § 436.325 of this chapter, except prepare the sample solution and calculate the vidarabine content as follows:

(i) *Preparation of sample solution.* Accurately weigh a portion of the sample containing the equivalent of approximately 12 milligrams of vidarabine (estimated) into a 100-milliliter volumetric flask. Add approximately 80 milliliters of distilled water and heat for 15 minutes on a steam bath. Shake to dissolve the vidarabine and, while the solution is still hot, add 10 milliliters of heptane to dissolve the ointment base. Swirl gently until the ointment base is dissolved. Cool to room temperature and dilute the aqueous phase to volume with distilled water. Discard the heptane phase and mix the solution.

(ii) *Calculations.* Calculate the vidarabine content as follows:

$$\text{Percent vidarabine} = \frac{A \times W_s \times f}{(B \times W_u \times 10)}$$

where:

$A$=Area of the vidarabine sample peak (at a retention time equal to that observed for the standard);
$B$=Area of the standard peak;
$W_s$=Weight of standard in milligrams;
$W_u$=Weight of sample in milligrams; and
$f$=Potency of standard in micrograms per milligram.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Metal particles.* Proceed as directed in §436.206 of this chapter.

[42 FR 44224, Sept. 2, 1977, as amended at 44 FR 30335, May 25, 1979; 50 FR 19921, May 13, 1985]

## Subpart E—Otic Dosage Forms

### §455.410 Chloramphenicol otic.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chloramphenicol otic is a solution of chloramphenicol in a suitable and harmless vehicle. Each milliliter contains 5.0 milligrams of chloramphenicol. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of chloramphenicol that it is represented to contain. It is sterile. Its moisture content is not more than 2 percent. Its pH is not less than 4 and not more than 8. The chloramphenicol used conforms to the standards prescribed by §455.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain the following:

(i) Results of tests and asays on—

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity; and

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using the sample diluted with an equal volume of distilled water.

[44 FR 5881, Jan. 30, 1979, as amended at 48 FR 3961, Jan. 28, 1983; 50 FR 19921, May 13, 1985]

## Subpart F—Dermatologic Dosage Forms

### §455.510 Chloramphenicol dermatologic dosage forms.

### §455.510a Chloramphenicol ointment (chloramphenicol cream).

The requirements for certification and the tests and methods of assay for chloramphenicol ointment (chloramphenicol cream) are described in §455.310c.

### §455.510b [Reserved]

### §455.510c Chloramphenicol-polymyxin ointment.

The requirements for certification and the tests and methods of assay for chloramphenicol-polymyxin ointment are described in §455.310d.

### § 455.510d Fibrinolysin and desoxyribonuclease, combined (bovine) with chloramphenicol ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Fibrinolysin and desoxyribonuclease, combined (bovine) with chloramphenicol ointment is fibrinolysin, desoxyribonuclease, and chloramphenicol in a suitable and harmless ointment base. It contains a suitable and harmless preservative. Each gram contains 1 unit of fibrinolysin, 666 units of desoxyribonuclease, and 10 milligrams of chloramphenicol. Its chloramphenicol content is satisfactory if it is not less then 90 percent and not more than 120 percent of the number of milligrams of chloramphenicol that it is represented to contain. The chloramphenicol used conforms to the standards prescribed by § 455.10, except paragraph (b)(2) of that section. In addition to the requirements prescribed by this paragraph, the drug satisfies the requirements designated therefor by the Center for Biologics Evaluation and Research, Food and Drug Administration, Department of Health and Human Services.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chloramphenicol used in making the batch for potency, pH, specific rotation, melting range, absorptivity, and crystallinity.

*(b)* The batch for potency.

(ii) Samples required:

(*a*) The chloramphenicol used in making the batch: 10 packages each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 5 containers if it is packaged in immediate containers of tin or glass, and a minimum of 20 immediate containers if it is packaged in immediate containers other than tin or glass.

(b) *Tests and methods of assay; potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of petroleum ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of distilled water and shake well. Allow the layers to separate. Remove the aqueous layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of distilled water. Combine the aqueous extractives in a suitable volumetric flask and dilute to volume with distilled water. Remove an aliquot and further dilute with distilled water to the reference concentration of 2.5 micrograms of chloramphenicol per milliliter (estimated).

[44 FR 10380, Feb. 20, 1979, as amended at 48 FR 3961, Jan. 28, 1983; 50 FR 19921, May 13, 1985; 55 FR 11585, Mar. 29, 1990]

### § 455.540 Mupirocin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Mupirocin ointment is mupirocin in a suitable and harmless ointment base. Each gram of ointment contains 20 milligrams of mupirocin. Its mupirocin content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of mupirocin that it is represented to contain. It passes the identity test. The mupirocin used conforms to the standards prescribed by § 455.40(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The mupirocin used in making the batch for potency, moisture, pH, crystallinity, and identity.

(B) The batch for mupirocin content and identity.

(ii) Samples, if required by the Center for Drug Evaluation and Research:

(A) The mupirocin used in making the batch: 10 packages, each containing not less than 300 milligrams.

(B) The batch: A minimum of 10 immediate containers.

(b) *Tests and methods of assay*—(1) *Mupirocin content.* Proceed as directed in § 455.40(b)(1), preparing the sample

solution and calculating the mupirocin content as follows:

(i) *Sample solution.* Accurately weigh approximately 0.5 gram of ointment and dissolve in 20 milliliters of acetonitrile. Transfer to a 100-milliliter volumetric flask with the aid of pH 6.3 phosphate buffer. Dilute to volume with pH 6.3 phosphate buffer. Mix well. The sample solution contains approximately 100 micrograms of mupirocin per milliliter (estimated).

(ii) *Calculations.* Calculate the mupirocin content in milligrams per gram as follows:

$$\text{Milligrams of mupirocin per gram} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times n}$$

where:

$A_u$=Area of the mupirocin peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

$A_s$=Area of the mupirocin peak in the chromatogram of the mupirocin working standard;

$A_s$=Mupirocin activity in the mupirocin working standard solution in micrograms per milliliter;

$d$=Dilution factor of the sample; and

n=Number of grams of sample assayed.

(2) *Identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(1) of this section compares qualitatively to that of the mupirocin working standard.

[55 FR 2642, Jan. 26, 1990]

# PART 460—ANTIBIOTIC DRUGS INTENDED FOR USE IN LABORATORY DIAGNOSIS OF DISEASE

## Subpart A—Susceptibility Discs

Sec.



## Subpart B—Susceptibility Powders



## Subpart C—Susceptibility Test Panels